FORM PTO-1390 (REV 11-2000)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTORNEY'S DOCKET NUMBER 39-252
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5) 10/018467 (To Be Assigned)
INTERNATIONAL APPLICATION NO. **PCT/GB00/02217**	INTERNATIONAL FILING DATE **19 June 2000**	PRIORITY DATE CLAIMED **19 June 1999**

TITLE OF INVENTION

ANTIBIOTIC AGENTS

APPLICANT(S) FOR DO/EO/US

BARBER et al

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The U.S. has been elected by the expiration of 19 months from the priority date (Article 31).
5. A copy of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   - b. ☒ has been communicated by the International Bureau.
   - c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U.S.C. 371(c)(2)).
   - a. ☐ is attached hereto.
   - b. ☐ has been previously submitted under 35 U.S.C. 154(d)(4).
7. ☐ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. ☐ are attached hereto (required only if not communicated by the International Bureau).
   - b. ☐ have been communicated by the International Bureau.
   - c. ☐ have not been made; however, the time limit for making such amendments has **NOT** expired.
   - d. ☐ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. ☐ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. ☐ A English language translation of the annexes of the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11 To 20 below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 C.F.R. 1.97 and 1.98.
12. ☐ An assignment document for recording. A separate cover sheet in compliance with 37 C.F.R. 3.28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment.
14. ☐ A SECOND or SUBSEQUENT preliminary amendment.
15. ☐ A substitute specification.
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C. 1.821-1.825.
18. ☐ A second copy of the published international application under 35 U.S.C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4).
20. ☒ Other items or information. PTO-1449 Form

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/018467 (To Be Assigned)	PCT/GB00/02217	39-252

21. ☒ The following fees are submitted:

	CALCULATIONS PTO USE ONLY
BASIC NATIONAL FEE (37 C.F.R. 1.492(a)(1)-(5):	
-- Neither international preliminary examination fee (37 C.F.R. 1.482) nor international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO $1040.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO $890.00	
-- International preliminary examination fee (37 C.F.R. 1.482) not paid to USPTO but international search fee (37 C.F.R. 1.445(a)(2)) paid to USPTO $740.00	
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO but all claims did not satisfy provisions of PCT Article 33(1)-(4) $710.00	
-- International preliminary examination fee (37 C.F.R. 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4) $100.00	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 890.00
Surcharge of $130.00 for furnishing the oath or declaration later than ☐ 20 ☒ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(e)).	$ 130.00

CLAIMS	NUMBER FILED		NUMBER EXTRA	RATE		
Total Claims	18	-20 =	0	X	$18.00	$ 0.00
Independent Claims	5	-3 =	2	X	$84.00	168.00
MULTIPLE DEPENDENT CLAIMS(S) (if applicable)					$280.00	$ 0.00

TOTAL OF ABOVE CALCULATIONS =	$ **1188.00**
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by 1/2.	0.00
SUBTOTAL =	$ **1188.00**
Processing fee of $130.00, for furnishing the English Translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 C.F.R. 1.492(f)). +	0.00
TOTAL NATIONAL FEE =	$ **1188.00**
Fee for recording the enclosed assignment (37 C.F.R. 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 C.F.R. 3.28, 3.31). **$40.00** per property +	$ 0.00
Fee for Petition to Revive Unintentionally Abandoned Application ($1280.00 – Small Entity = $640.00)	$ 0.00
TOTAL FEES ENCLOSED =	$ **1188.00**
	Amount to be: refunded $
	Charged $

a. ☒ A check in the amount of $1188.00 to cover the above fees is enclosed.
b. ☐ Please charge my Deposit Account No. 14-1140 in the amount of $_____ to cover the above fees. A duplicate copy of this form is enclosed.
c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 14-1140. A duplicate copy of this form is enclosed.
d. ☒ The entire content of the foreign application(s), referred to in this application is/are hereby incorporated by reference in this application.

NOTE: Where an appropriate time limit under 37 C.F.R. 1.494 or 1.495 has not been met, a petition to revive (37 C.F.R. 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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32,955 December 19, 2001
REGISTRATION NUMBER Date

586355

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

BARBER et al — Atty. Ref.: 39-252

National Phase of Int'l Appln. No. PCT/GB00/02217 — Group:

International Filing Date: 19 June 2000 — Examiner:

For: ANTIBIOTIC AGENTS

* * * * * * * * * * *

December 19, 2001

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

PRELIMINARY AMENDMENT

In order to place the above-identified application in better condition for examination, please amend the application as follows:

IN THE CLAIMS

Please substitute the following amended claims for corresponding claims previously presented. A copy of the amended claims showing current revisions is attached.

5. (Amended) An antibiotic composition as claimed in claim 1 which contains 50mg to 1g of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

7. (Amended) An antibiotic composition as claimed in claim 1 in the form of a tablet, a capsule, a suspension, an elixir, a syrup or an injectable.

9. (Amended) A pharmaceutical composition as claimed in claim 1 containing a pharmaceutically acceptable derivative of Erythromycin B and where said derivative is a 2'-ester of Erythromycin B or of Erythromycin B enol ether.

12. (Amended) An ester as claimed in claim 11 which is a succinate ester.

13. (Amended) An antibiotic composition comprising a therapeutically effective amount of an ester as claimed in claim 10.

17. (Amended) The use as claimed in claim 14 in which the microbial infection is TB, Syphilis, Helicobacter pylori, or Chlamydia.

Add the following new claim.

18. (New) The method as claimed in claim 15 in which the microbial infection is TB, Syphilis, Helicobacter pylori, or Chlamydia.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page(s) is captioned "**Version With Markings To Show Changes Made**."

Respectfully submitted,

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VERSION WITH MARKINGS TO SHOW CHANGES MADE

IN THE CLAIMS

5. (Amended) An antibiotic composition as claimed in [any one of claims 1 to 4] claim 1 which contains 50mg to 1g of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

7. (Amended) An antibiotic composition as claimed in [any one of claims 1 to 6] claim 1 in the form of a tablet, a capsule, a suspension, an elixir, a syrup or an injectable.

9. (Amended) A pharmaceutical composition as claimed in [any one of claims 1 to 8] claim 1 containing a pharmaceutically acceptable derivative of Erythromycin B and where said derivative is a 2'-ester of Erythromycin B or of Erythromycin B enol ether.

12. (Amended) An ester as claimed in claim [12] 11 which is a succinate ester.

13. (Amended) An antibiotic composition comprising a therapeutically effective amount of an ester as claimed in [any one of claims 10 to 12] claim 10.

17. (Amended) The use as claimed in [any one of claims 13, 14 or 15] claim 14 in which the microbial infection is TB, Syphilis, Helicobacter pylori, or Chlamydia.

ANTIBIOTIC AGENTS

The present invention relates to antibiotic agents and more particularly such agents which overcome disadvantages associated with erythromycin A.

This specification comprises Figs. 1 to 23 of drawings. Of these, Figs. 1 and 2 comprise Formulae Sheets incorporating the formulae to which reference is made below.

The actinomycete *Saccarropolyspora erythra* produces erythromycin A (1) and several of its biosynthetic precursors (erythromycins B-F). Erythromycin A is one of the most widely used and important antibacterial agents in current clinical use. The other erythromycins, most notably erythromycin B, are also antibacterial agents[1] of varying potency, but are not used in clinical practice.

Erythromycin A, despite its clinical utility, has several undesirable properties. It has a vile taste, causing a degree of poor compliance in children. No amount of work on paediatric formulations has yet successfully masked the taste. It and its degradation products cause severe gastric disturbance in sensitised adults, leading again to poor compliance. The drug and its degradation products are processed by cytochrome P450 enzymes in the liver, which are also responsible for processing other drugs including common hay fever remedies. Erythromycin A and its by-products are able to saturate these enzymes, so that the blood levels of the other drugs rise, leading to overdose.

All of these problems arise in large part because erythromycin A is extremely acid sensitive. It has a half-life of about an hour at pH 4.6 and 60°C[2]. Thus large doses (up to 2g per day) are required for efficacy, because a high proportion of the administered drug is degraded in the acid conditions of the stomach. One of the products of erythromycin A degradation is the spiroketal as shown in the decomposition pathway illustrated in Fig. 3[3,4] of the accompanying drawings. The

degradation products do not have antibacterial activity, but they do cause gastric disturbance and are metabolised in the liver.

In the last few years, two derivatives of erythromycin A have appeared in the clinic. They are clarithromycin (2) and azithromycin (3). Both of these compounds are made from erythromycin A but have the disadvantages of requiring several synthetic steps for their production. Clarithromycin is unable to cyclise in the 6,9 direction because the 6-hydroxy group is methylated. Azithromycin is unable to cyclise at all because C9 has been modified. Both are acid-sensitive, but much less so than erythromycin A. Typical recommended dose rates are 500mg per day (divided into two doses) for 5 days (clarithromycin) and 500mg per day (in one or two doses) for 3-5 days (azithromycin), rather than typically 1g to 2g per day administered in 4 doses for 7-12 days as with erythromycin A. It is reasonable to regard both of these compounds as "acid-stable erythromycin A". The degradation pathway for these compounds has been explored and involves expulsion of the cladinose sugar in both cases (Fig. 4).[5,6]

The present invention seeks to obviate or mitigate the abovementioned disadvantages by providing alternative antibacterial agents to Erythromycin A, clarithromycin and azithromycin.

According to one aspect of the present invention there is provided Erythromycin B (or a pharmaceutically acceptable derivative thereof) for use in therapeutic administration for the treatment of microbial (e.g. bacterial) infection

We have found that Erythromycin B (4), a biosynthetic precursor of erythromycin A, has advantages over erythromycin A, clarithromycin and azithromycin. *In vitro* it has similar antibacterial activity to erythromycin A.[1] It lacks the 12-hydroxy function and therefore cannot cyclise in a 12,9 direction; (12-9 cyclisation is the first step of acid-catalysed degradation) It is not overly simplistic to regard erythromycin B as a mirror image of clarithromycin. We have carried out

degradation studies under acid conditions on erythromycin B and shown that (i) the mechanism of degradation involves loss of the cladinose sugar in the same way as clarithromycin and azithromycin and (ii) that the drug is much more stable than erythromycin A. Its stability is comparable with that of clarithromycin and azithromycin.

Erythromycin B (or derivative) has the further advantage as an acid-stable antibiotic in that it is a fermentation product and therefore avoids the synthetic steps employed in the manufacture of clarithromycin and azithromycin. Furthermore, the vile taste common to the macrolide antibiotics can be readily masked in the case of Erythromycin B and its derivatives using existing inexpensive technology.

Erythromycin B is currently not used in clinical practice but our stability studies indicate that it would be a very suitable replacement for Erythromycin A, clarithromycin and azithromycin in many circumstances. Thus, Erythromycin B (or derivative) may be used for treating a microbial (e.g. bacterial) infection by administering to a patient in need of such treatment a therapeutically effective amount of Erythromycin B (or derivative). In view of its enhanced stability compared to Erythromycin A, Erythromycin B (or derivative) may be used in lower dosage rates. Erythromycin B (or derivative) may be used in an amount up to 500 mg (e.g. 250 to 500 mg) per day as compared to 2 g per day as generally employed for Erythromycin A.

According to another aspect of the present invention, there is provided an antibiotic composition comprising at least 50% by weight of Erythromycin B (or a pharmaceutically acceptable derivative thereof) based on the total weight of antibiotic present in the composition.

Preferably the antibiotic composition comprises at least 75% by weight of Erythromycin B (or derivative), most preferably at least 90%, on the same basis as defined in the previous paragraph.

Suitably the composition will contain only Erythromycin B (or derivative) as antibiotic.

The composition of the invention may contain between 50mg and 1g, preferably 100mg to 500mg Erythromycin B (or derivative).

The composition will incorporate a pharmaceutically acceptable vehicle and may be in any suitable form known in the art, suitably tablet, capsule, suspension, elixir, syrup or as an injectable. A tablet containing 250mg or 500mg of Erythromycin B (or derivative thereof) is most preferred.

In the manufacture of paediatric formulations, erythromycin A and its derivatives are normally esterified at the 2' position to give compounds such as erythromycin A ethyl succinate (5). Similar derivatives of clarithromycin have not yet appeared.

In accordance with a further aspect of the present invention there are provided 2'-ester derivatives of Erythromycin B or of Erythromycin B enol ether. The invention also provides an antibiotic formulation comprising a therapeutically effective amount of such an ester, preferably in conjunction with a pharmaceutically acceptable vehicle.

The esters may be derived, for example, from any mono- or dicarboxylic acid. A preferred example of a mono- or dicarboxylic acid is succinic acid, which leads to the formation of a succinate mono- or di- ester. The ester may be used for the treatment of microbial (e.g. bacterial) infections by administering a therapeutically effective amount of the ester to a patient in need of treatment.

The administration of the 2'-ester derivatives may be as described above for administration of Erythromycin B and its derivatives. Thus, for example, the 2'-ester

may be provided in a pharmaceutical composition containing at least 50% by weight of the 2'-ester based on the total weight of antibiotic in the composition.

All of the active agents disclosed herein may be used for a treatment of a wide range of microbial (e.g. bacterial) infections. Examples include TB, Syphilis, Helicobacter pylori, or Chlamydia. The agents may alternatively or additionally be used for the treatment of any condition for which a penicillin would normally be employed and have advantages in that they may be used for the treatment of penicillin sensitive patients

The invention is illustrated by way of example only by the following Experimental Section and accompanying Figures 5 to 23 of the drawings.

Experimental section

(i) Background

The effective buffer range for a weak acid and base is approximately from pH=pKa+1 to pH=pKa-1 (a range of 2 pH units). The buffering ability increases (a wider pH) range when two or more buffers are present (additive effects). Furthermore, if a buffer system has several successive pKa values which differ by 2 pH units, approximately linear buffer capacity could be obtained (Perrin & Dempset, 1987). Several universal buffers have been established such as citric acid-phosphate buffer for pH2.6-8 (McIlvaine, 1921), piperazine-glycylglycine buffer for pH 4.4-10.8 (Smith & Smith, 1949) and Britton-Robinson buffer for pH2.6-12 (Britton & Robinson, 1931).

Alam (1996) has shown that phosphate and acetate buffers are not suitable for low pH studies. He used Britton-Robinson buffer pH2, as the medium for the degradation of Azithromycin. It is a universal buffer, relatively inexpensive and its deuteriated components are readily available, or can be prepared in the laboratory. It

is convenient to use deuteriated buffer species in the NMR application as they are lacking in protons.

In our studies, samples of erythromycin B (4mM) were prepared in deuteriated Britton-Robinson buffer (4mM) at apparent pH 2.5. This initial degradation experiment has been set up to form the buffering capacities of 4mM Britton-Robinson buffer at pH 2.5. The apparent pHs of samples were determined over time. It was found that the apparent pH of 2.5 was maintained during the course of the degradation. The results suggest that the Britton-Robinson buffer is giving adequate buffering to the system at lower pH. This finding is consistent with the fact that Britton-Robinson has been widely used as a versatile buffer, buffering a system as low as pH 0.3 and as high as pH 13 (Barroso et al., 1995).

(ii) Degradation Studies of Erythromycin B

Almost all drugs are subject to some form of degradation. In pharmaceutical applications, it is a standard procedure to study the degradation of medicinal compounds at extreme temperature and then extrapolate the information to other temperature conditions. Where NMR technique is used, degradation studies should be completed in a reasonable time period, because of the expense of instrument time. As a set of spectra is required to generate a good degradation profile of any particular compound, conditions should be established so that the degradation occurs at a reasonable rate yet not too fast, as a good noise-to-signal ratio needs a few minutes to be accomplished. In this experiment, spectra were initially measured every 5 min. Corresponding to 32 scans pulsing every 6 sec.

Several degradation studies involving erythromycin A have been carried out at different buffers, ionic strength, temperatures and pHs employing HPLC-UV technique (Cachet et al., 1989; Atkins et al., 1986) and NMR spectroscopy measurement (Alam et al., 1996). All the studies showed that the rate of decomposition was very dependent on the pH and temperature.

Erythromycin A in 50 mM formate buffer pH 4 decomposed almost completely within 4 hours at 37°C (Atkinson et al, 1986). Another study showed that erythromycin A in 50 mM acetate buffer pH 4.6, degraded at 60°C to completeness within 3 hours (Alam, 1996). Furthermore, by reducing the pH and temperature to 3.5 and 35°C respectively, erythromycin A degraded substantially even before the first spectrum was recorded (Alam, 1996). Based on those observations and the fact that erythromycin B lacks the hydroxyl group at position 12 which is believed to be important for its better stability, pH 4.6 was chosen as the initial pH in the degradation of erythromycin B. It was found that, at pH 4.6, degradation occurred very slowly and it was not sensible to monitor its rate of degradation. Further studies showed that decomposition of erythromycin B at pH 2.5 was completed in reasonable time periods depending on the temperature used (6-12 hr).

A full kinetics decomposition of erythromycin B study was carried out in Britton-Robinson buffer pH 2.5 at temperatures of 35, 40, 45, 50 and 55°C. Initially, array 1D ^{1}H NMR spectra were acquired either every 5 min, corresponding to 32 scans pulsing every 6 sec and dummy scans of 18 (for 45, 50 and 55°C experiments) acquired every 10 min, corresponding to 32 scans pulsing every 10 sec with dummy scans of 28 (for 35, 40 and 45°C experiments). Once the experiment had proceeded for an hour, NMR spectra were acquired less frequently by increasing the numbers of dummy scans used.

The 1D ^{1}H spectrum of erythromycin B at pH 2.5 showed distinct peaks pattern when compared to the spectrum of fully degraded samples which were later identified as 5-desosaminyl erythronolide B (5-deB). The differences in the spectra were characterised by the distinctive coupling constants and chemical shifts of signals in erythromycin B and 5-deB which could be used to monitor the degradation of erythromycin B. The distinct peaks whose disappearance in erythromycin B and the appearance in 5-deB could be monitored comfortably were signals of H13, H1' and H5"; the chemical shifts changed from δ5.29 to δ5.40, δ4.60 to δ4.97 and δ4.44 to

δ4.73, respectively. The typical 1D^{1}H NMR spectra of erythromycin B and 5-deB in Britton-Robinson buffer, apparent pH 2.5, are depicted in Figures 5 and 6 respectively.

A degradation profile of erythromycin B was constructed by observing the disappearance of signal H13 at δ5.29, whilst for the product (5-deB) accumulation, H13 signal at δ5.40 was chosen. A set of degradation spectra at 55°C is presented in Figure 7. From the spectra, it is clearly observed that H13 of erythromycin B decreased over the time, while at the same time the H13 of 5-deB increased. The degradation profile was then plotted by using the integral values of erythromycin B and 5-deB, and this profile is depicted in Figure 8. TSP was used as the marker for consistency of the degradation experiment as its integral value did not change during the degradation course and its peak also did not interfere with the signals of the compounds.

As shown in Figure 8, the concentration of erythromycin B decreased exponentially whilst the concentration of 5-deB shows fairly similar increase in the magnitude. However, when the mass of both compounds was added together, the total mass was not maintained all the time. In the first portion of the degradation course, the total mass of erythromycin B and 5-deB increased over time and was followed by a plateau. The same pattern of degradation was also demonstrated at 35 and 45°C and the profile is depicted in Figure 9. These results suggest, at the beginning of degradation reaction, a mass balance was not conserved due to the existence of the third compound which participates in the reaction. This intermediate compound was later identified as erythromycin B enol ether (eBee).

(iii) Deuteriation Effect at C8

During the degradation course of erythromycin B, deuteriation at C8 (H8 has been replaced with deuterium atom, D8) has been observed in the 1D^{1}H spectra. Thus, the $H_3$19 which appeared initially as a doublet at δ1.17 has been transformed to

a singlet at δ1.16. A set of spectra demonstrating the deuteriation of erythromycin B at 40°C is shown in Figure 10. By using an electrospray-mass spectrometry (EM-MS) technique, this effect was clearly observed and a typical MS chromatogram showing the peaks of erythromycin B (MW 717), erythromycin B-D (MW 718) and the degradation product, 5-deB (MW 559) is presented in Figure 11. (Note that the actual mass of every compound should be less 1 mass unit from the values shown in the spectrum). Therefore, a deuteriation and/or degradation profile of erythromycin B at 55°C based on ES-MS study was plotted and is shown in Figure 12. This plot suggests that at time zero (apparent degradation should not occur), some erythromycin has already been deuteriated and its amount was increased over time and the appearance rate of both erythromycin B-D and 5-deB were quite similar.

At this point, it is difficult to postulate whether the deuteriation involves the degradation of erythromycin B to 5-deB, or if it is just an instantaneous reaction between erythromycin B and its deuteriated buffer.

(iv) Identification of Degradation Product

In this study we employed an NMR technique (DOSY, Diffusion Ordered Spectroscopy). With DOSY, a mixture of molecules of very similar size can be readily analysed. As such, this technique is a powerful alternative to physical separation technique for the analysis of complex mixtures such as HPLC.

Erythromycin B was degraded completely and the degradation sample was run in the NMR spectrometer employing the ^{1}H-DOSY experiment. A typical DOSY spectrum of degradation sample is presented in Figure 13. Most of the blobs in the DOSY spectrum are aligned into two rows suggesting there were 2 compounds in the mixture. These blobs could be characterised by the chemical shift of 5-deB. The first row, consisting of the most blobs, is the erythromycin B ring with desosamine sugar still attached to it. Another row of blobs suggest that cladinose sugar has fallen away

from the erythromycin B ring. A ROESY (Rotating frame nuclear Overhauser Effect Spectroscopy) experiment was used to support the results.

The ROESY spectrum of erythromycin B and a degraded sample of erythromycin B, are presented in Figures 14 and 15, respectively. In Figure 14, there are cross-peaks between H1" (in cladinose molecule) to H3 and H16 (in erythromycin B ring), and between H1' (in desosamine molecule) to H5 and H17 (in erythromycin B ring). Cross-peaks occur in this spectrum when two protons are within 5 Angstrom units of each other. In Figure 15, there are no cross-peaks between H1" to any protons in the erythromycin B ring, however, there are cross-peaks between H1' of desosamine molecule, to protons in the erythonolide B ring. These observations are in agreement to the results of DOSY experiment where cladinose ring has fallen away from the erythromycin B ring, but the desosamine sugar is still intact. Degradation of cladinose has also been reported for erythromycin A analogues such as clarithromycin (Nakagawa et al., 1992) and azithromycin (Fiese & Steffen, 1990) but does not occur to erythromycin A itself (Alan, 1996; Cachet et al., 1989; Atkins et al., 1989). Mass spectrometry was used to obtain the molecular weight of every species in the degradation mixture. A typical mass spectrum of the degradation product of erythromycin B is depicted in Figure 16. (Note that the actual mass of every compound should be less 1 mass unit from the values shown in the spectrum).

(v) Verification that 5-desosaminyl Erythromycin (5-deB) is the End Product

The 5-deB sample in Britton-Robinson buffer, apparent pH 2.5, was degraded at 55°C for 5 hr and a set of 1D ^{1}H spectra was acquired and is depicted in Figure 17, whilst Figure 18 shows the downfield 0.80-1.35 ppm region of the spectra. The deuteriation effects at C8 as in erythromycin B were observed but there was no change elsewhere. We postulate that 5-deB does not degrade further under this condition (pH 2.5, 55°C, 5 hr degradation period).

(vi) Identification of Erythromycin B Enolether as the Intermediate in the Degradation Reaction of Erythromycin B

In the degradation profile of erythromycin B to 5-deB, it was found that the mass balance was not consistent all the time due to the existence of the other compound(s) (section ii). We believe that erythromycin B enol ether (eBee) takes role as the intermediate in the degradation reaction of erythromycin B, similar to the involvement of erythromycin A enol ether during the decomposition of erythromycin A.

The suspected intermediate, eBee was prepared and then was added into a partially degraded erythromycin B, the mixture of partially degraded erythromycin B, and the pure eBee solution were compared. The singlet signal of H19 at δ1.60 could be used to demonstrate the existence of eBee. However, the experiment was not successful because eBee at pH2.5 was rapidly degraded to erythromycin B and could not be seen in the spectrum. However, the involvement of eBee as the intermediate in the degradation of erythromycin B was clearly observed in the degradation experiment of eBee.

(vii) Degradation of Erythromycin B Enol ether

Two mechanisms of degradation were proposed for erythromycin A. Atkins and associates (1986) suggested erythromycin A decomposed to anhydroerythromycin A via erythromycin A enol ether (see Figure 1). Cachet et al. (1989) postulated that during decomposition erythromycin A is in equilibrium with erythromycin A enol ether and simultaneously erythromycin A is directly converted to anhydroerythromycin A (see Figure 2). The mechanism proposed by Cachet is consistent with the work of Perwaiz (1996) where he concluded that erythromycin A enol ether is degraded to anhydroerythromycin A via erythromycin A.

At present, there is no mechanism of degradation of erythromycin B which has been proposed. An experiment was designed to distinguish whether eBee is degraded to 5-deB via erythromycin B or not. Britton-Robinson buffers, 40 mM of pH2 and 7 were prepared and aliquoted into eppendorf tubes. Just before the experiment started, erythromycin B was dissolved in 20ul of dimethyl-d_6 sulfoxide (99.9 atom % D, Sigma, St Louis, USA) and then was transferred immediately into the buffer and 1D ^{1}H NMR spectra were recorded at 37°C. The typical spectra at 0, 10 and 20 min are depicted in Figures 19, 20 and 21, respectively.

The spectra suggested that at pH2, eBee, characterised by signal H19 at δ1.59, was initially existing together with erythromycin B, identified by its H13 at δ5.29, However, no 5-deB was detected in the spectrum. After 10 min, the amount of eBee was reduced to almost 40%, but 5-deB was still undetectable. After 20 min, 5-deB appeared in the spectrum, characterised by its H13 at δ5.38, whilst eBee became insignificant in the spectrum. Spectra at 30 min and onwards demonstrating a typical degradation of erythromycin B where the amount of erythromycin B decreased whilst the amount of 5-deB increased over time.

A similar experiment was repeated by using buffer at pH 7, and 1D ^{1}H spectra were recorded at 37°C. The spectrum of eBee after 0 and 60 min of incubation are depicted in Figures 22 and 23, respectively. The spectra at pH7 showed that eBee was not degraded at least for 80 min where the experiment had been carried out. There was trace of erythromycin B but no eBee was detected in the spectra.

Both results at pH2 and 7 suggested that eBee in a solution can be converted to erythromycin B, and subsequently, erythromycin B can be converted to 5-deB. This reaction is very much dependent on the acidity of the environment. However, the actual mechanism of reaction is still not clear.

For example, by comparing the degradation rate of eBee and erythromycin B to 5-deB, we could possibly say that eBee degrades via erythromycin B, if eBee

degrades at least at the same rate as erythromycin B or maybe slower. The degradation mechanism below could be suggested:

$$\text{eBee} \leftrightarrow \text{erythromycin B} \rightarrow \text{5-deB}$$

However, if eBee degrades at least the same rate as erythromycin B or faster, we could suggest that erythromycin B degrades via eBee as shown below:

$$\text{erythromycin B} \leftrightarrow \text{eBee} \rightarrow \text{5-deB}$$

So far, we are unable to postulate the reaction mechanism of erythromycin B. However, the results suggest that eBee could possibly be a prodrug of erythromycin B. In acid environment of the stomach, eBee will be converted to erythromycin B and depending on the gastric emptying time, erythromycin B could possibly survive decomposition and enter the intestine as an intact form where it could be absorbed into the blood circulation.

References

1 IO Kibwaga, J. Hoogmartens, E. Roets, H. Vanderharghe, L. Verbist, M. Dubost, C. Pascal, P. Petitjean and G. Levol, Antimicrob. Agents Chemother. 1985, 28, 630.

2 P.S. Alam, Ph.D. Thesis, University of Manchester, 1997.

3 PJ Atkins, TO Herbert and NB Jones, Int. J. Pharm. 1986, 30, 199.

4 T. Cachet, GV Mooter, R. Hauchecorne, C. Vinckier and J. Hoogmartens, Int. J. Pharm. 1989, 55, 59.

5 EF Fiese and SH Steffen, J. Antimicrob. Chemother. 1990, 25 (suppl. A), 39.

6 Y. Nakagawa, S. Itai, T. Yoshida and T. Nagai, Chem. Pharm. Bull. (Japan) 1992, 40, 725.

Alam, P.S., in "*The study of drug degradation using nuclear magnetic resonance spectroscopy*", PhD Thesis, dept. Pharmacy, Univ. Manchester, 1996.

Alam, P.S., Buxton, P.C., Parkinson, J.A. and Barber, J., *J. Chem. Soc. Perkin. Trans. 2.*, 1163, 1995.

Amer, M.M. and Takla, K.F., *Bull. Faculty Pharmacy Cairo University*, **15**, 325, 1978.

Atkins, P.J., Herbert, T.O. and Jones, N.B., *Int. J. Pharmaceutics*, **30**, 199, 1986.

Atkins, P.W., et al., in "*Principles of Physical Chemistry*", Pitman, London, 1983.

Barber, J., Gyi, J.I., Lian, L., Morris, G.A., Pye, D.A. and Sutherland, J.K., *J. Chem. Soc. Perkin. Trans. 2.*, 1489, 1991.

Barber, J., Gyi, J.I., Lian, L., Morris, G.A., Pye, D.A. and Sutherland, J.K., *J. Chem. Soc. Chem. Comm.*, 1040, 1990.

Barroso, M.B., Alonso, R.M. and Jimenez, R.M., *Anal. Chim. Acta.*, 305, 332, 1995.

Bell, R.P., in "*Acid-base Catalysis*", Oxford University Press, London, 1941.

Brittain, D.P., *Med. Clin. N. Am.*, **71**, 1147, 1987.

Cachet, T.H., Van den Mooter, G., Hauchecorne, R., Vinckier, C. and Hoogmartens, J., *Int. J. Pharm.*, **55**, 59, 1989.

Carestensen, J.T., in "*Drug stability: Prnciples and Practises*", Marcel Dekker, New York, 11, 1990.

Contreras, J.T. and Vasquez, D., *Eur. J. Biochem.*, **74**, 539, 1977.

Fiese, E.F. and Steffen, S.H., *J. Antimicrob. Chemother.*, 25 (Suppl A), 39, 1990.

Ghebre-Sellassie, I., Hem, S.L. and Knevel, A.M., *J. Pharm. Sci.*, **73**, 125, 1984.

Hinselwood, C.N., "*The Kinetics of Chemical Change*", Oxford University Press, London, 1940.

Hollis, D.P., *Anal. Chem.*, **35**, 1682, 1963.

Inatomi, N., Satoh, H., Satoh, T., Itoh, Z. and Omura, S., Eur. *J. Pharmacol.*, **183**, 2183, 1990.

Janssens, J., Peeters, T.L., Vantrappen, G., Tack, J., Urbain, J.L., De Roo, M., Muls, E. and Bouillon, R., *N. Engl. J. Med.*, **322**, 1028, 1990.

Kim, S.K., Jung, M.Y. and Kim, S.Y., *Food. Chem.*, **59**, 273, 1997.

Kirst, H.A., in "*Progress in Medicinal Chemistry*", Ellis, G.P. and Luscombe, D.K. (Eds), Elsevier Science Publisher, Vol 30, 57, 1993.

Kirst, H.A., in "*Kirk-Othmer Encyclopedia of Chemical Technology*", 4th Ed, Wiley, New York, Vol 3, 169, 1992.

Kitamura, S., Tada, T., Okamoto, Y. and Yasuda, T., *Pharm. Res.*, **9**, 138, 1992.

Kondrat'eva, A.P. and Burns, B.P., *Antibiotik*, **7**, 571, 1962.

Krowicki, K. and Zamosjski, A., *J. Antibiotics.*, 26, 58, 1973.

Kucers, A. and Bennet, N.M. in "*The use of Antibiotics, A comprehensive review with clinical emphasis*", 4th Ed, Heineman, Oxford, 851, 1989.

Kurath, P. and Egan, R.S., *Hiv. Chim. Acta.*, **54**, 523, 1971.

Labeda, D.P., *Int. J. Syst. Bacteriol.*, **37**, 19, 1987.

Laidler, K.J., in "*Chemical Kinetics*", 2nd Ed., McGraw-Hill Book Co Inc., N.Y., 1965.

Lerner, D.A., Bonneford, G., Fabre, H., Mondru, B. and deBuochberg, M.S., *J. Pharm. Sci.*, **77**, 699, 1988.

McGuire, J.M., Bunch, R.L., Anderson, R.C., Boaz, H.E., Flynn, E.H., Powell, H.M. and Smith, J.W., *Antibiot. Chemother.*, **2**, 281, 1952.

Menninger, J.R. and Otto, D.P., *Antimicrob. Agents Chemother.*, **21**, 811, 1982.

Mitsumori, F., Arata, Y., Fujiwara, S. and Muranka, M., *Bull. Chem. Soc. Japan.*, **50**, 3164, 1977.

Moore, J.W. and Pearson, R.G., in "*Kinetics and Mechanism*", John Willy & Sons, N.Y., 1981.

Nakagawa, Y., Itai, S., Yoshida, T. and Nagai, T., *Chem. Pharm. Bull.*, 40, 725, 1992.

Omura, S., Tsuzuki, K., Sunazuka, T., Marui, S., Toyoda, H., Inatomi, N. and Itoh, Z., *J. Med. Chem.*, **30**, 1941, 1987.

Pye, D.A., Gyi, J.I., and Barber, J., *J. Chem. Soc. Chem. Comm.*, 1143, 1990.

Raez, I. in "*Drug Formulation*", John Willy & Sons, N.Y., 1989.

Ruckmick, S.C. and Duong, S.T., *J. Pharm. Sci.*, **84**, 502, 1995.

Stephen, V.C. and Conine, J.W.in "*Antibiotics Annual 1958-59*", Welch, H. and Marti-Ibanez, F. (Eds), Medical Encyclopedia Inc., 346, 1959.

Sunazuka, T., Tsuzuki, K., Marui, S., Toyoda, H., Omura, S., Inatomi, N. and Itoh, Z., *Chem. Pharm. Bull.*, **37**, 2701, 1989.

Tait-Kamradt, A., Clancey, J., Cronan, M., Dib-Hajj, F., Wondrack. L., Yuan, W. and Sutcliffe, J., *Antimicrob. Agents Chemother.*, 41, 2251, 1997.

Teraoka, H. and Nierhaus, K.H., *J. Mol. Biol.*, **126**, 185, 1978.

Tsuzuki, K., Sunazuka, T., Marui, S., Toyoda, H., Omura, S., Inatomi, N. and Itoh, Z., *Chem. Pharm. Bull.*, **37**, 2687, 1989.

Vinckier, C., Hauchecorne, R., Cachet, T., Van de Mooter, G. and Hoogmartens, J., *Int. J. Pharm.*, **55**, 67, 1989.

Visconti, M., Citerio, L., Borsa, M. and Pifferi, G., *J. Pharm. Sci.*, **73**, 1812, 1984.

Wang, D. and Notari, E., *J. Pharm. Sci.*, **83**, 577, 1974.

CLAIMS

1. An antibiotic composition comprising at least 50% by weight of Erythromycin B (or a pharmaceutically acceptable derivative thereof) based on the total weight of antibiotic present in the composition.

2. An antibiotic composition as claimed in claim 1 which contains at least 75% by weight of Erythromycin B (or a pharmaceutically acceptable derivative thereof) based on the total weight of antibiotic present in the composition.

3. An antibiotic composition as claimed in claim 2 which contains at least 90% by weight of Erythromycin B (or a pharmaceutically acceptable derivative thereof) based on the total weight of antibiotic present in the composition.

4. An antibiotic composition as claimed in claim 3 which contains Erythromycin B (or a pharmaceutically acceptable derivative thereof) as substantially the only antibiotic.

5. An antibiotic composition as claimed in any one of claims 1 to 4 which contains 50mg to 1g of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

6. An antibiotic composition as claimed in claim 5 which contains 100mg to 500mg of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

7. An antibiotic composition as claimed in any one of claims 1 to 6 in the form of a tablet, a capsule, a suspension, an elixir, a syrup or an injectable.

8. An antibiotic composition as claimed in claim 7 in the form of a tablet containing 250mg to 500mg of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

8. An antibiotic composition as claimed in claim 7 in the form of a tablet containing 250mg to 500mg of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

9. A pharmaceutical composition as claimed in any one of claims 1 to 8 containing a pharmaceutically acceptable derivative of Erythromycin B and where said derivative is a 2'-ester of Erythromycin B or Erythromycin B enol ether.

10. A 2'-ester of Erythromycin B or of Erythromycin B enol ether.

11. An ester as claimed in claim 10 wherein the ester is a carboxylate ester of a mono or dicarboxylic acid.

12. An ester as claimed in claim 12 which is a succinate ester.

13. An antibiotic composition comprising a therapeutically effective amount of an ester as claimed in any one of claims 10 to 12.

14. The use of Erythromycin B (or a pharmaceutically acceptable derivative thereof) for the manufacture of a pharmaceutical composition for the treatment of microbial infection.

15. A method of treating a microbial infection comprising administering to a patient in need of such treatment a therapeutically effective amount of Erythromycin B (or a pharmaceutically acceptable derivative thereof).

16. Erythromycin B (or a pharmaceutically acceptable derivative thereof) for use in therapeutic administration for the treatment of microbial infection.

17. A use or method as claimed in any one of claims 13, 14 or 15 in which the microbial infection is TB, Syphilis, Helicobacter pylori, or Chlamydia.

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
28 December 2000 (28.12.2000)

PCT

(10) International Publication Number
WO 00/78772 A2

(51) International Patent Classification[7]: C07H 17/00

(21) International Application Number: PCT/GB00/02217

(22) International Filing Date: 19 June 2000 (19.06.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
9914346.3 19 June 1999 (19.06.1999) GB

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(81) Designated States *(national)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

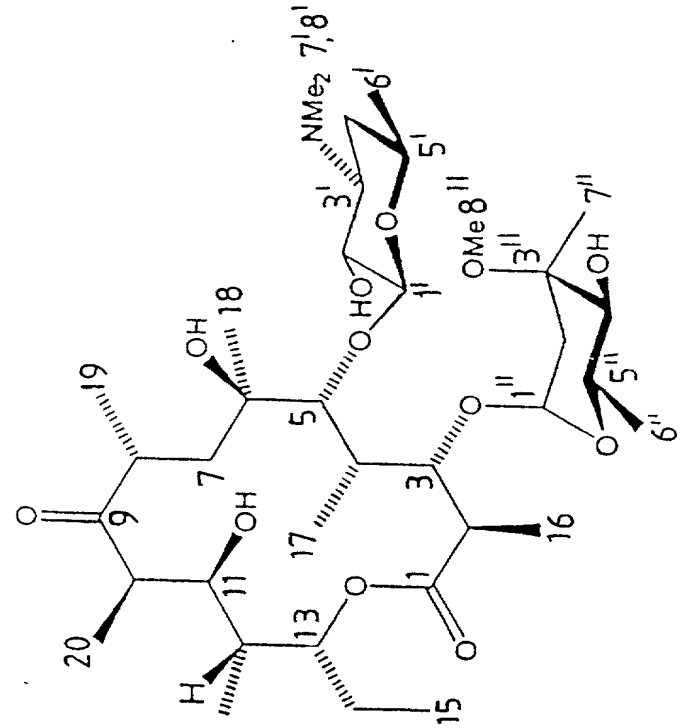
Published:
— *Without international search report and to be republished upon receipt of that report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

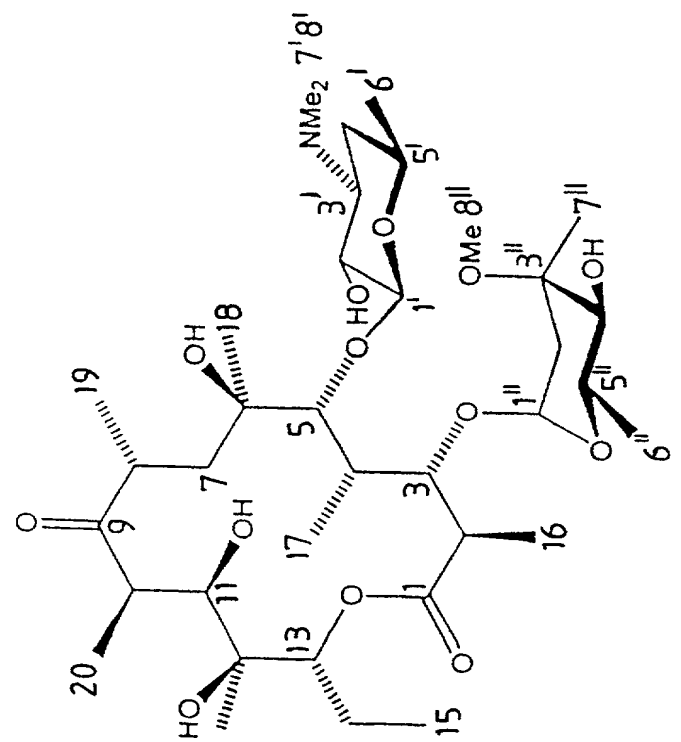
WO 00/78772 A2

(54) Title: ANTIBIOTIC AGENTS

(57) Abstract: Methods of treating a microbial infection comprising administering to a patient in need of such treatment are disclosed. In one aspect the method comprises administering to a patient in need of such treatment a therapeutically effective amount of Erythromycin B (or a pharmaceutically acceptable derivative thereof). In another aspect the method comprises a pharmaceutical composition comprising at least 50 % by weight of Erythromycin B (or a pharmaceutically acceptable derivative thereof) of the total weight of antibiotic present in the composition. Also disclosed are 2'-esters of Erythromycin B and Erythromycin B enol ether.

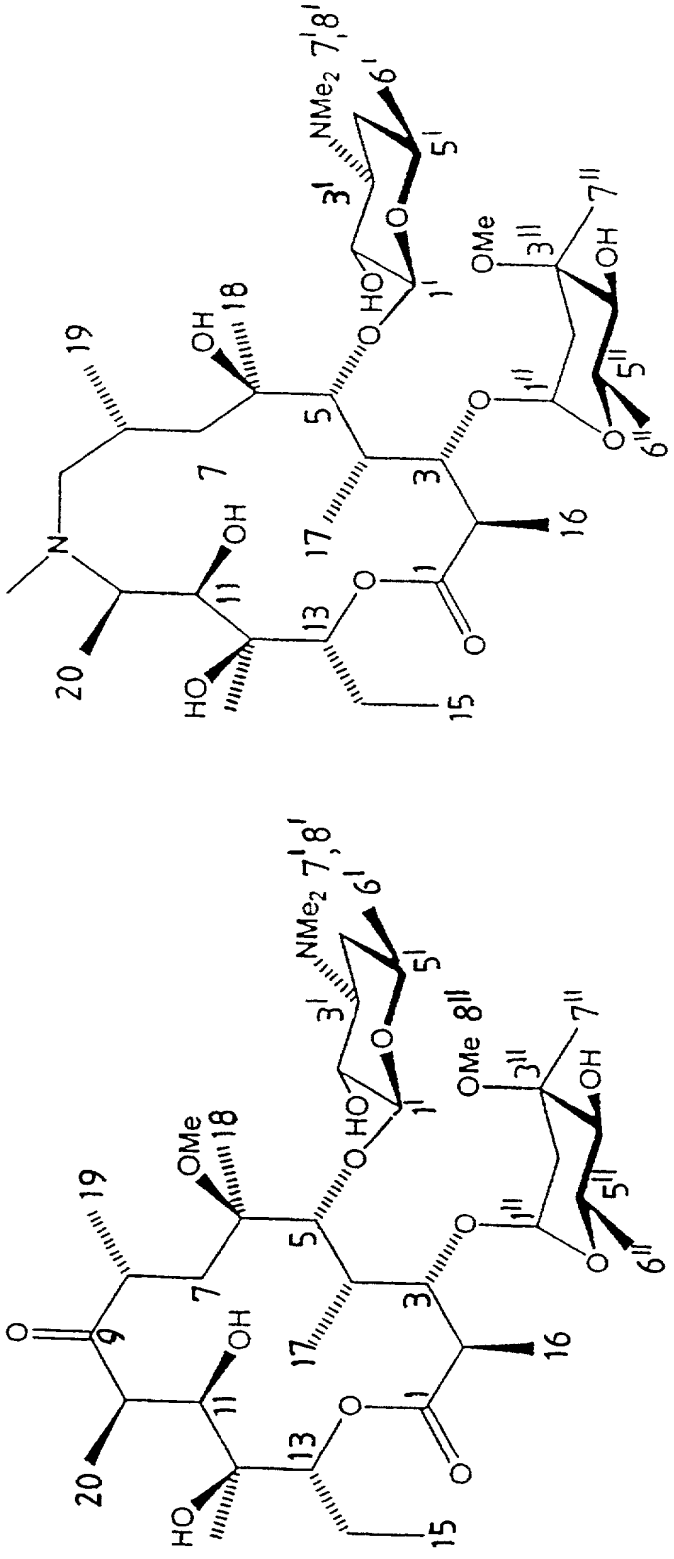


4 - Erythromycin B



1 - Erythromycin A

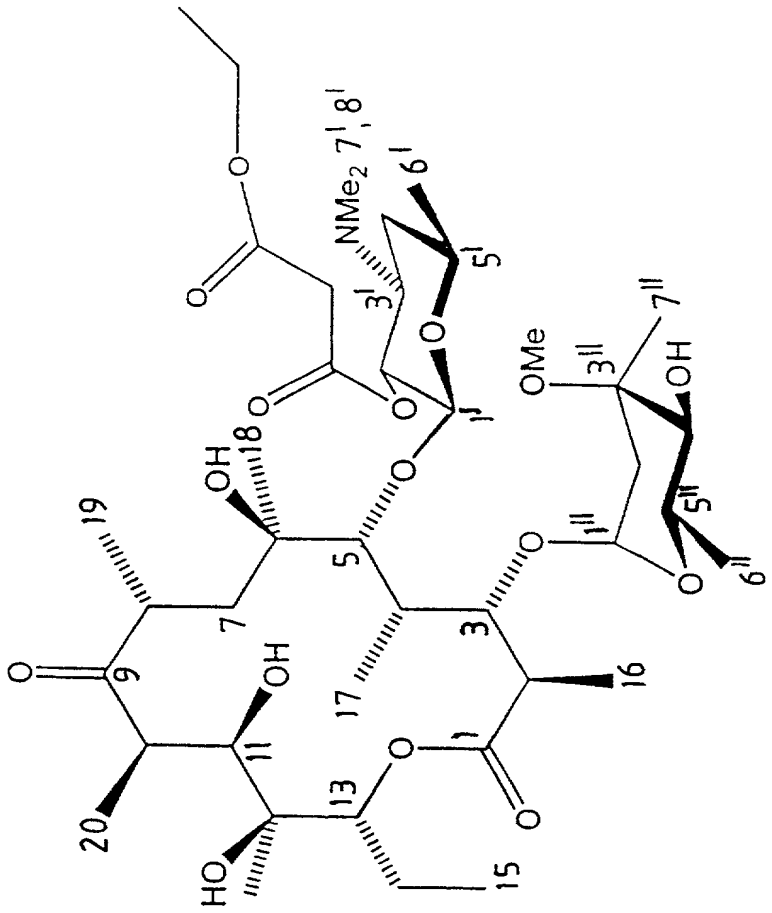
FIG. 1



2-Clarithromycin

3-Azithromycin

FIG. 1(cont)



5 – Erythromycin A ethyl succinate

FIG. 2

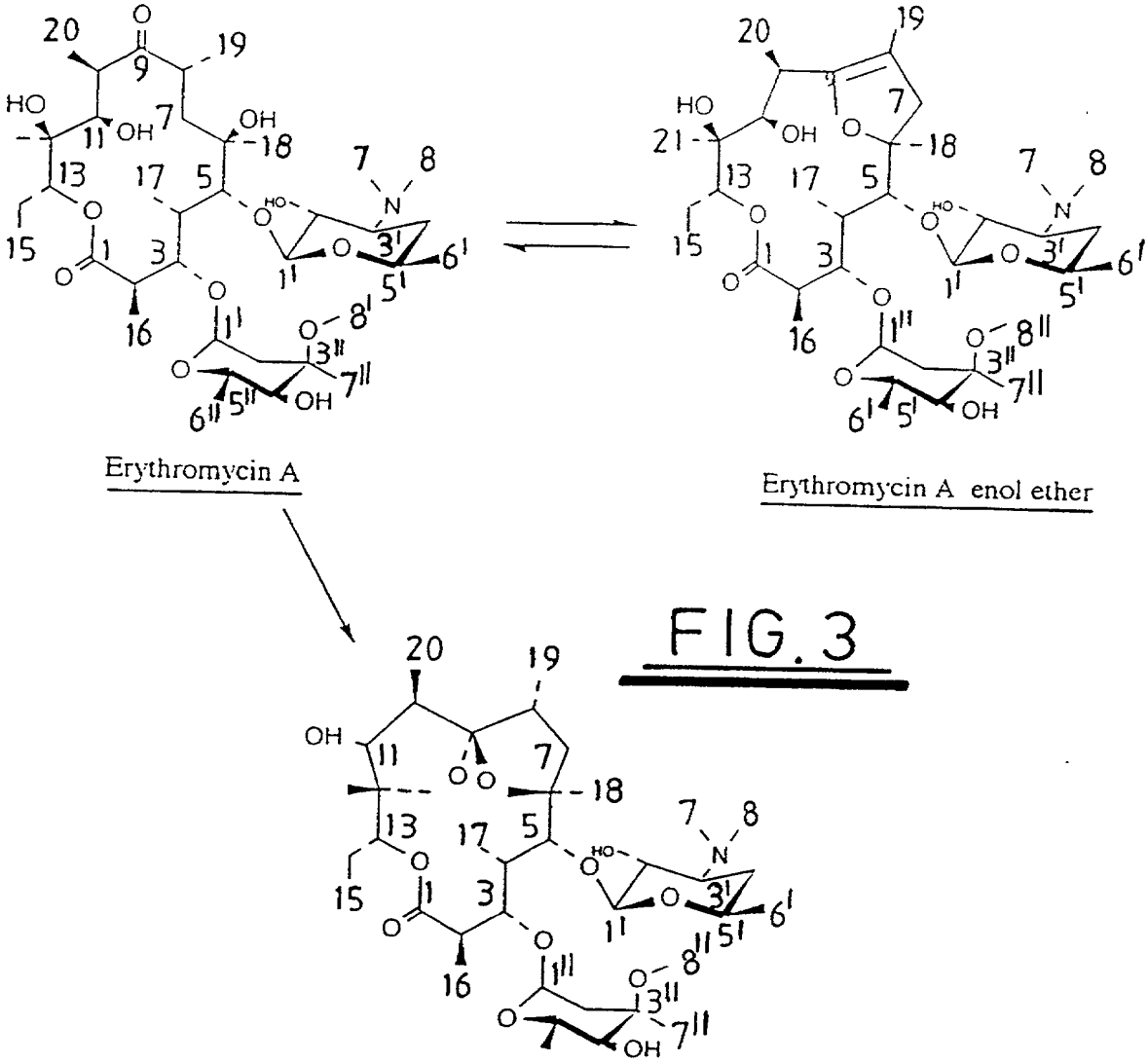


FIG. 3

Decomposition pathway for erythromycin A in aqueous acidic medium

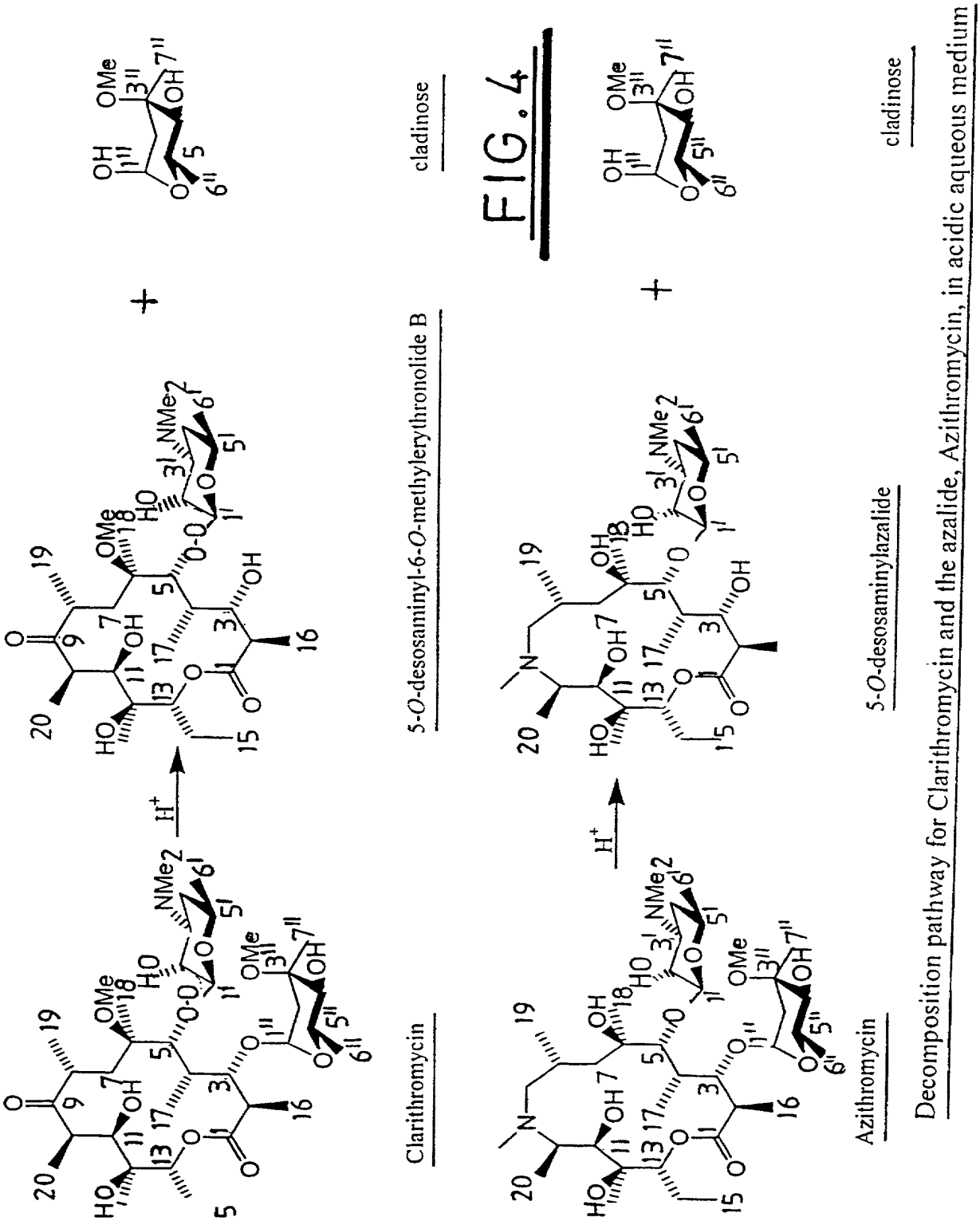


FIG. 4

Decomposition pathway for Clarithromycin and the azalide, Azithromycin, in acidic aqueous medium

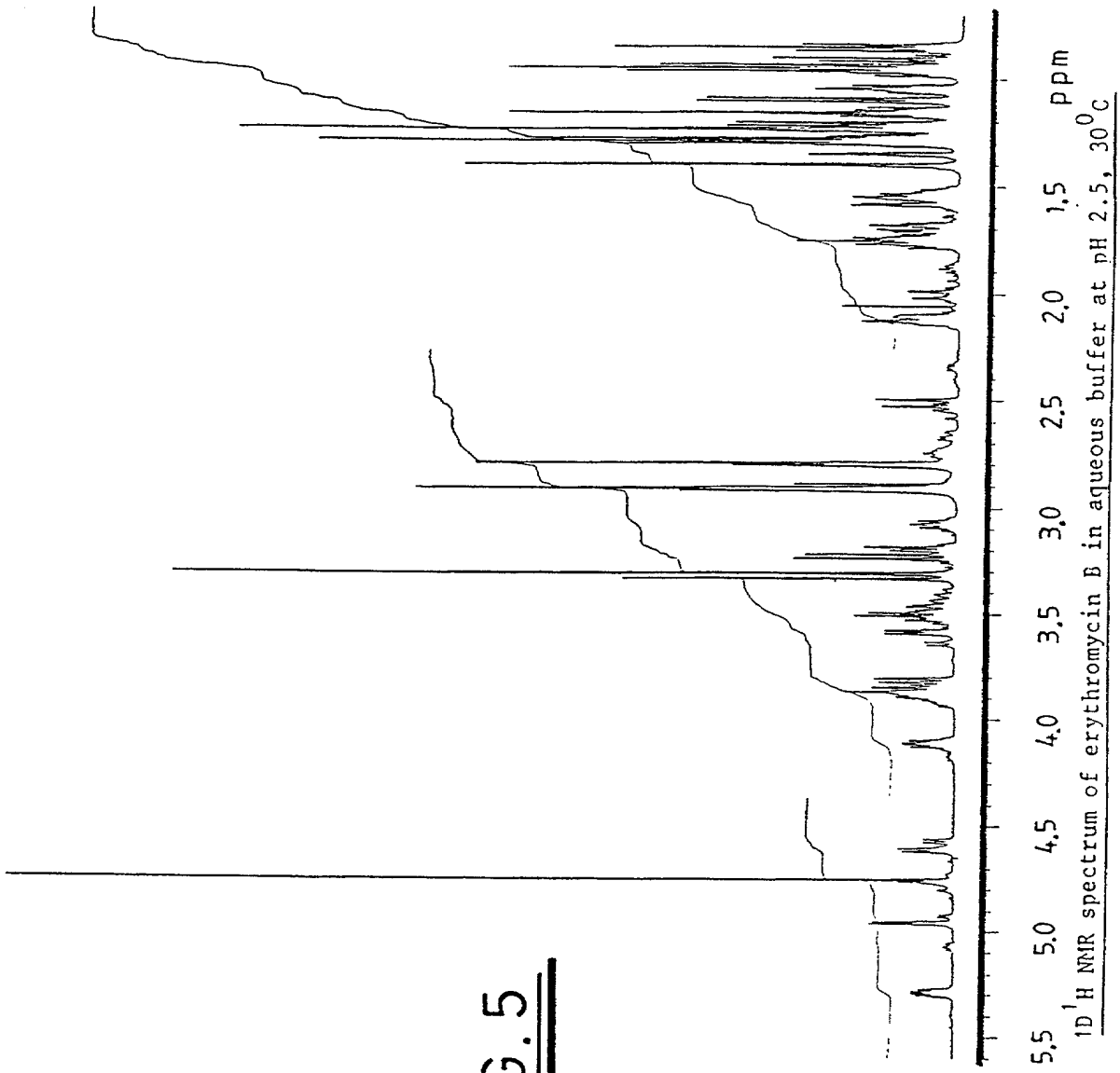

5,5
5.0
4.5
4.0
3,5
3,0
2,5
2,0
1,5
ppm
1D ^{1}H NMR spectrum of erythromycin B in aqueous buffer at pH 2.5, 30^{0}C

FIG. 5

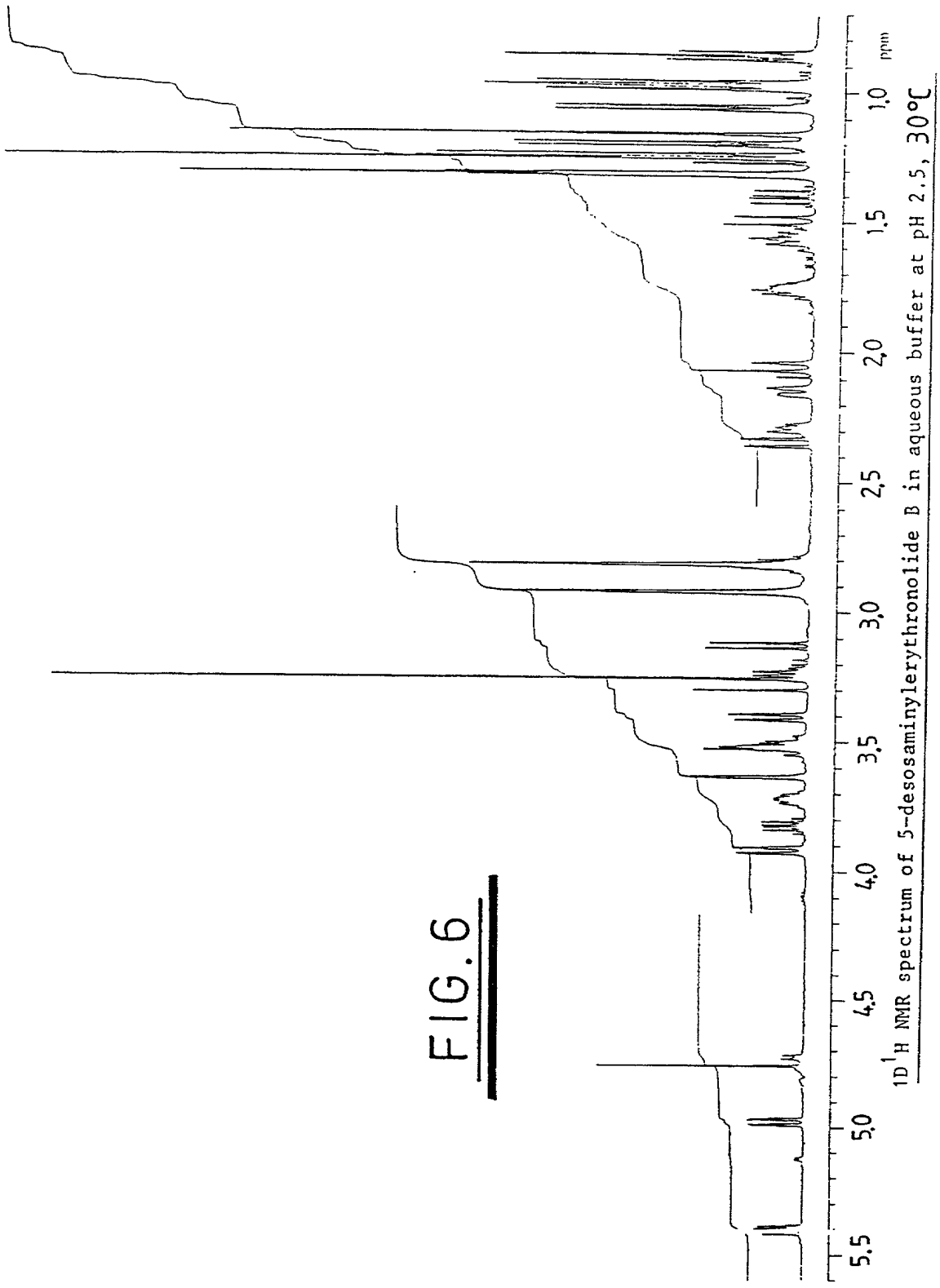

1D ^{1}H NMR spectrum of 5-desosaminylerythronolide B in aqueous buffer at pH 2.5, 30°C
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5

FIG.6

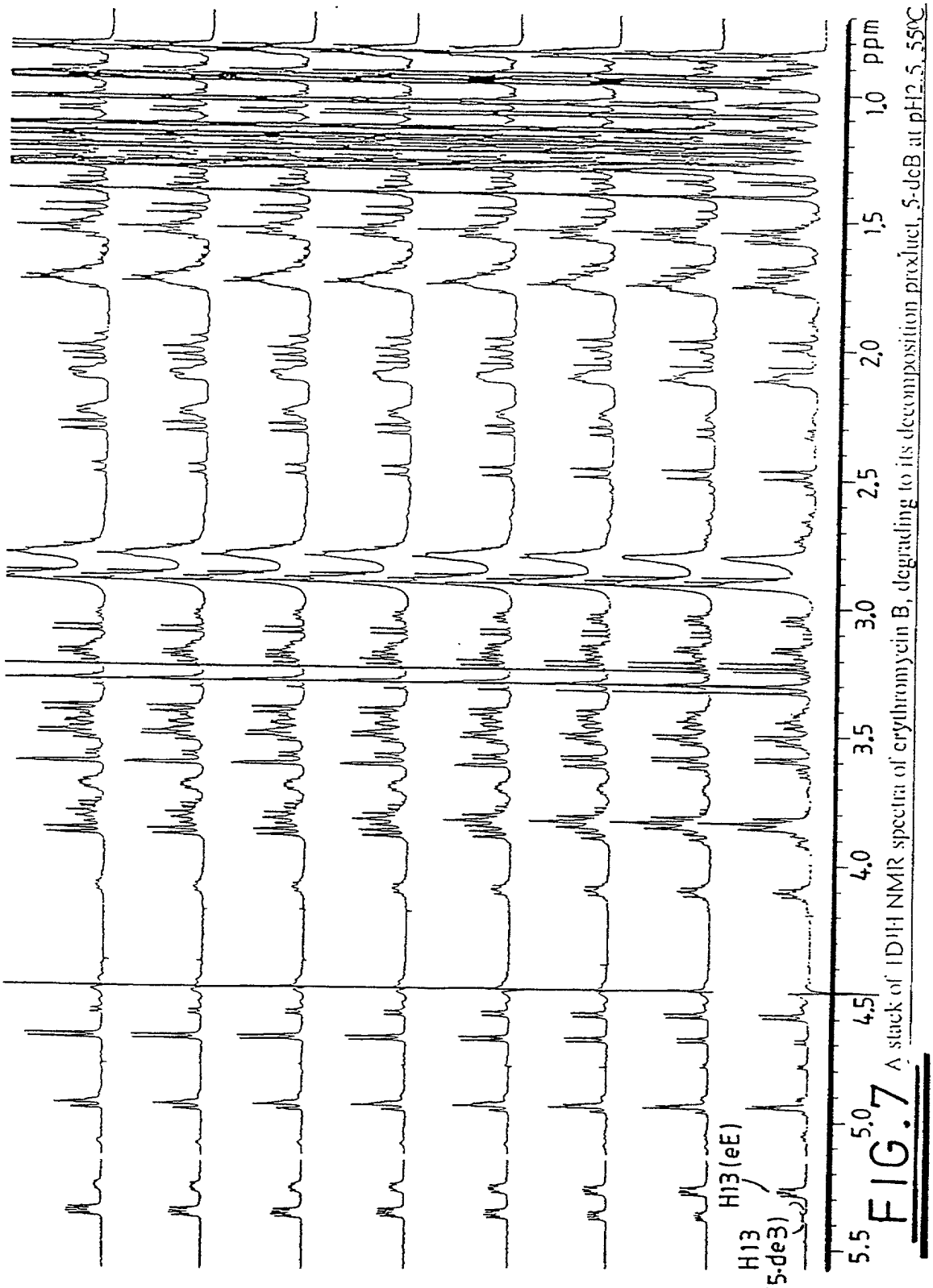


FIG. 7 A stack of 1DH NMR spectra of erythromycin B, degrading to its decomposition product, 5-deB at pH2.5, 55°C

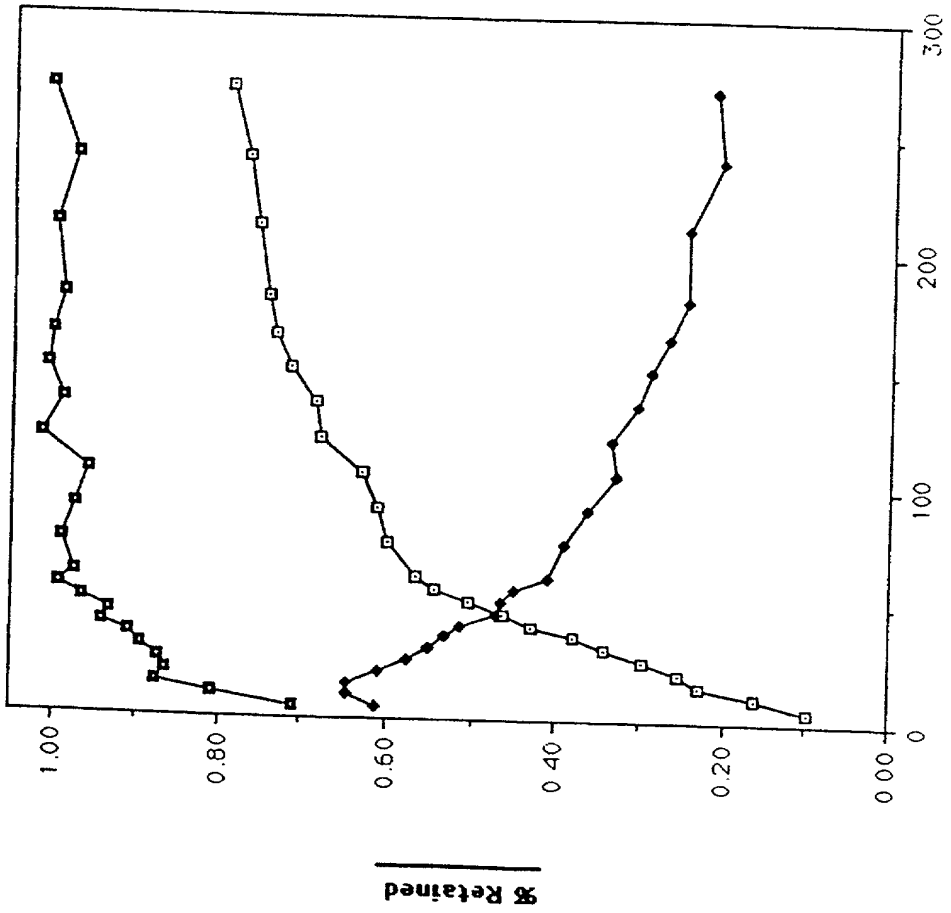
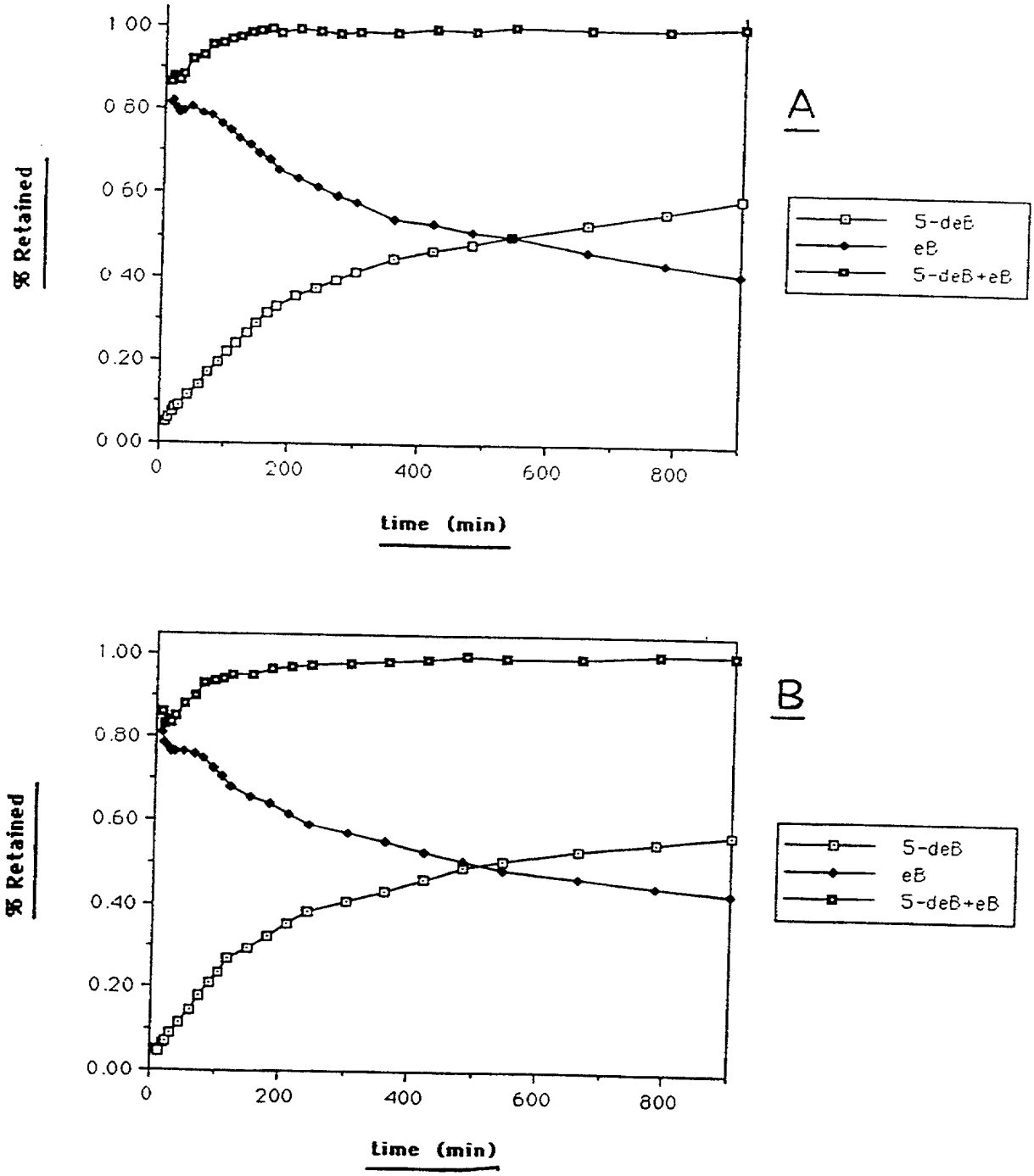


FIG. 8

Plot of percentage remained/accumulated in the degradation solution for erythromycin B (eB), 5-deB and the total amount of both erythromycin B and 5-deB (5-deB + eB) in Britton-Robinson buffer, pH 2.5, 55°C



Plot of percentage remained/accumulated in the degradation solution for erythromycin B (eB), 5-deB and the total amount of both erythromycin B and 5-deB (5-deB + eB) in Britton-Robinson buffer, pH 2.5, and 35°C (A) and 45°C (B)

FIG. 9

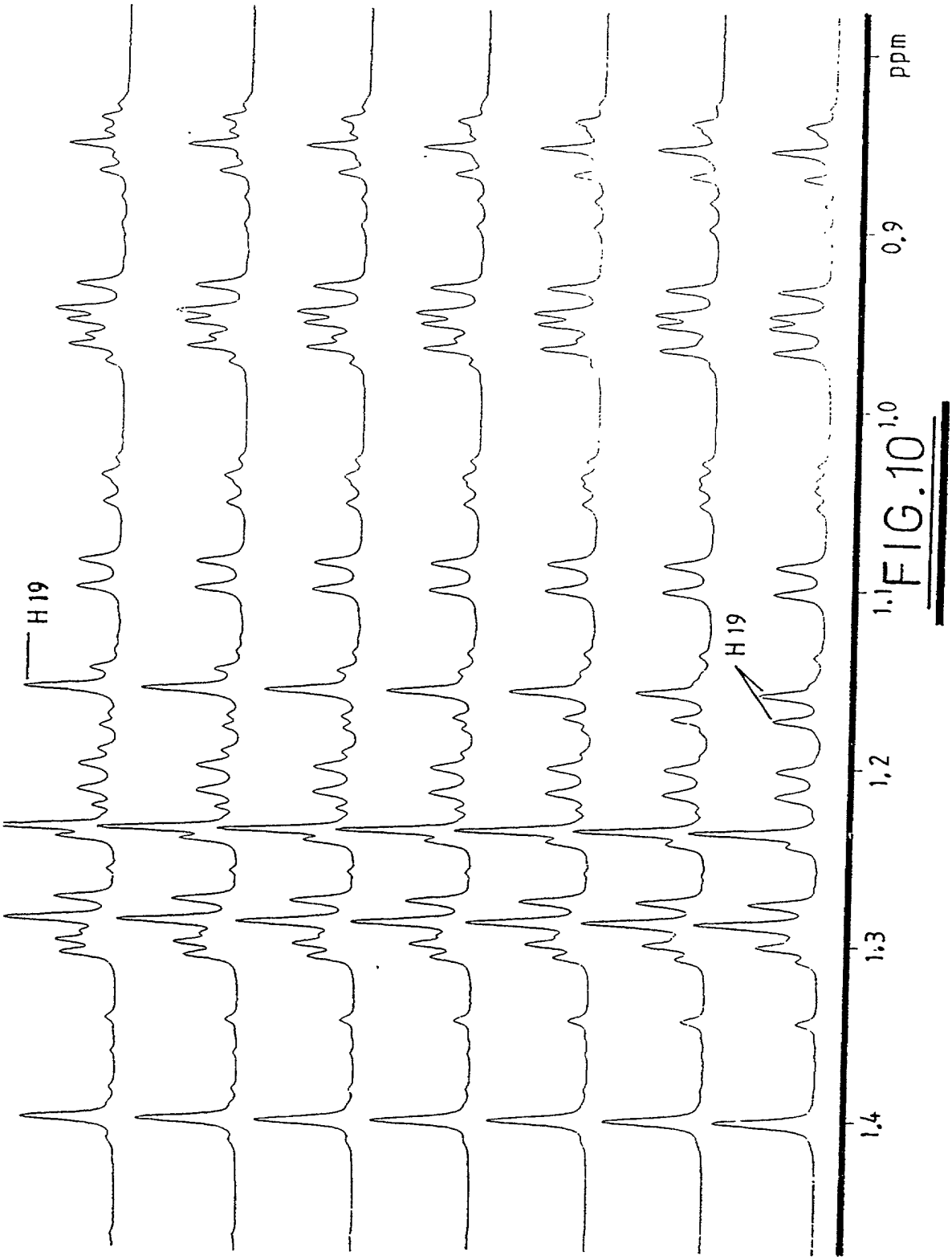
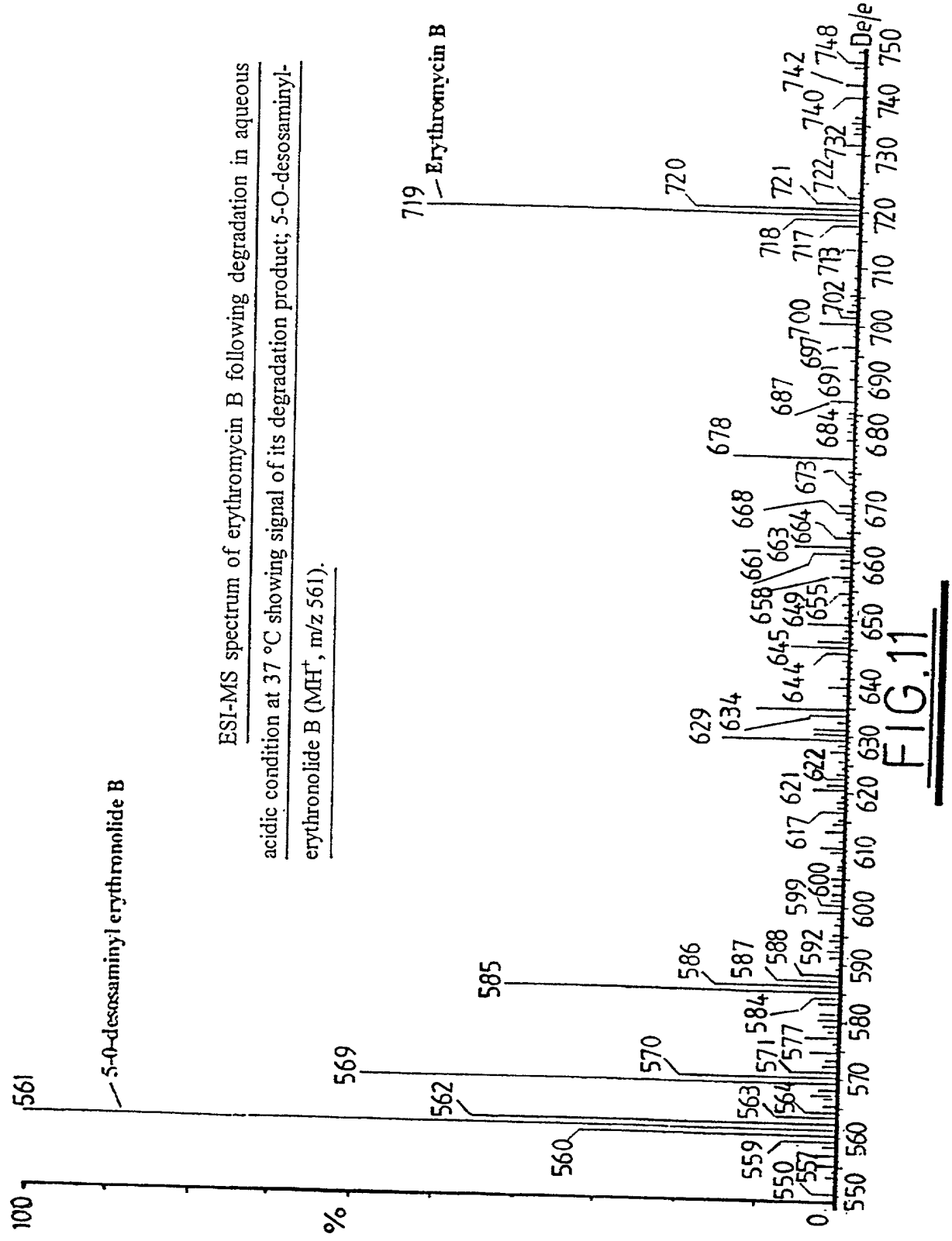


FIG.10



ESI-MS spectrum of erythromycin B following degradation in aqueous acidic condition at 37 °C showing signal of its degradation product; 5-O-desosaminyl-erythronolide B (MH^+, m/z 561).

FIG.11

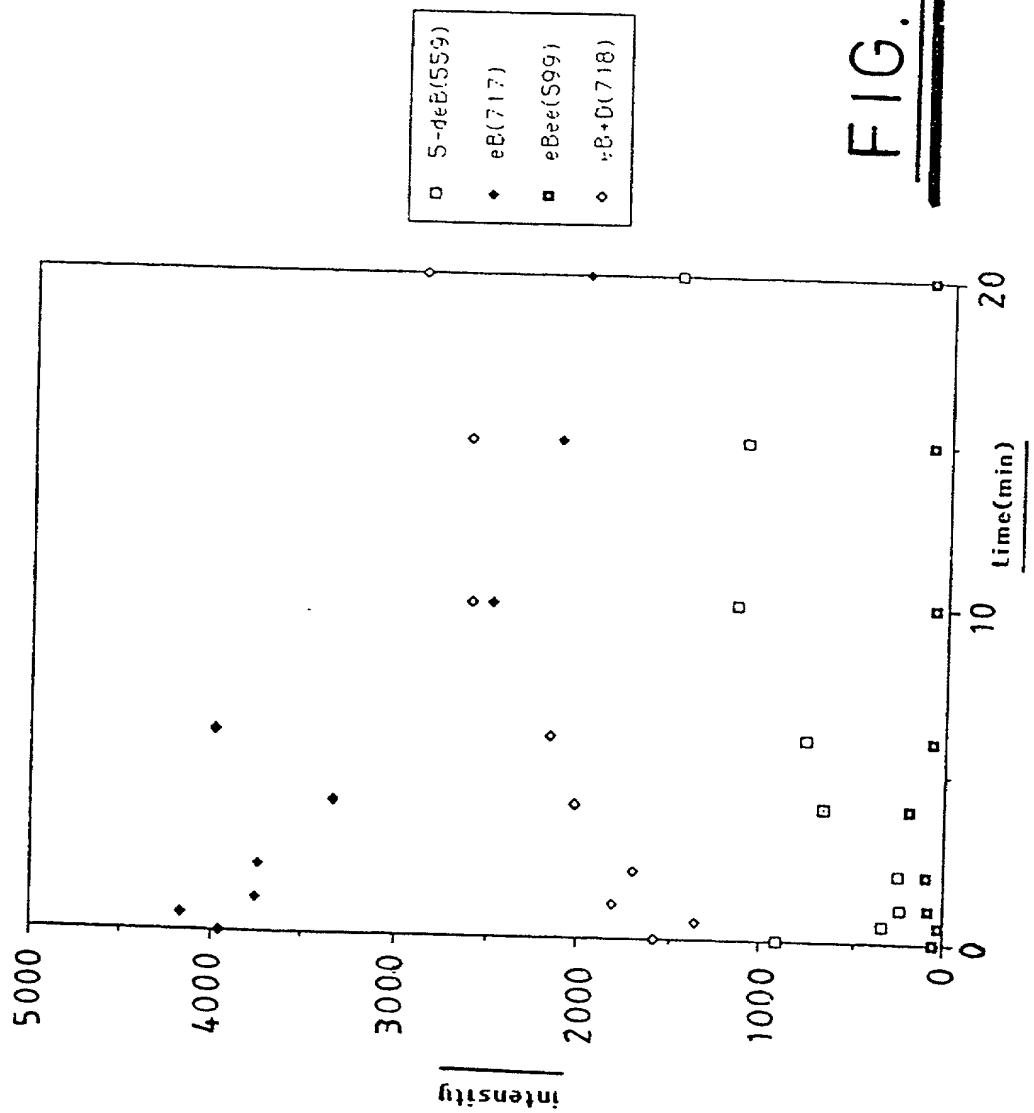


FIG.12

Plot of intensity of erythromycin B (eB, MW 717), erythromycin B Deutron (eB+D, MW 718), eBec (MW 599) and 5-deB (MW 559) during the degradation of erythromycin B to 5-deB at pH 2.5, 55C

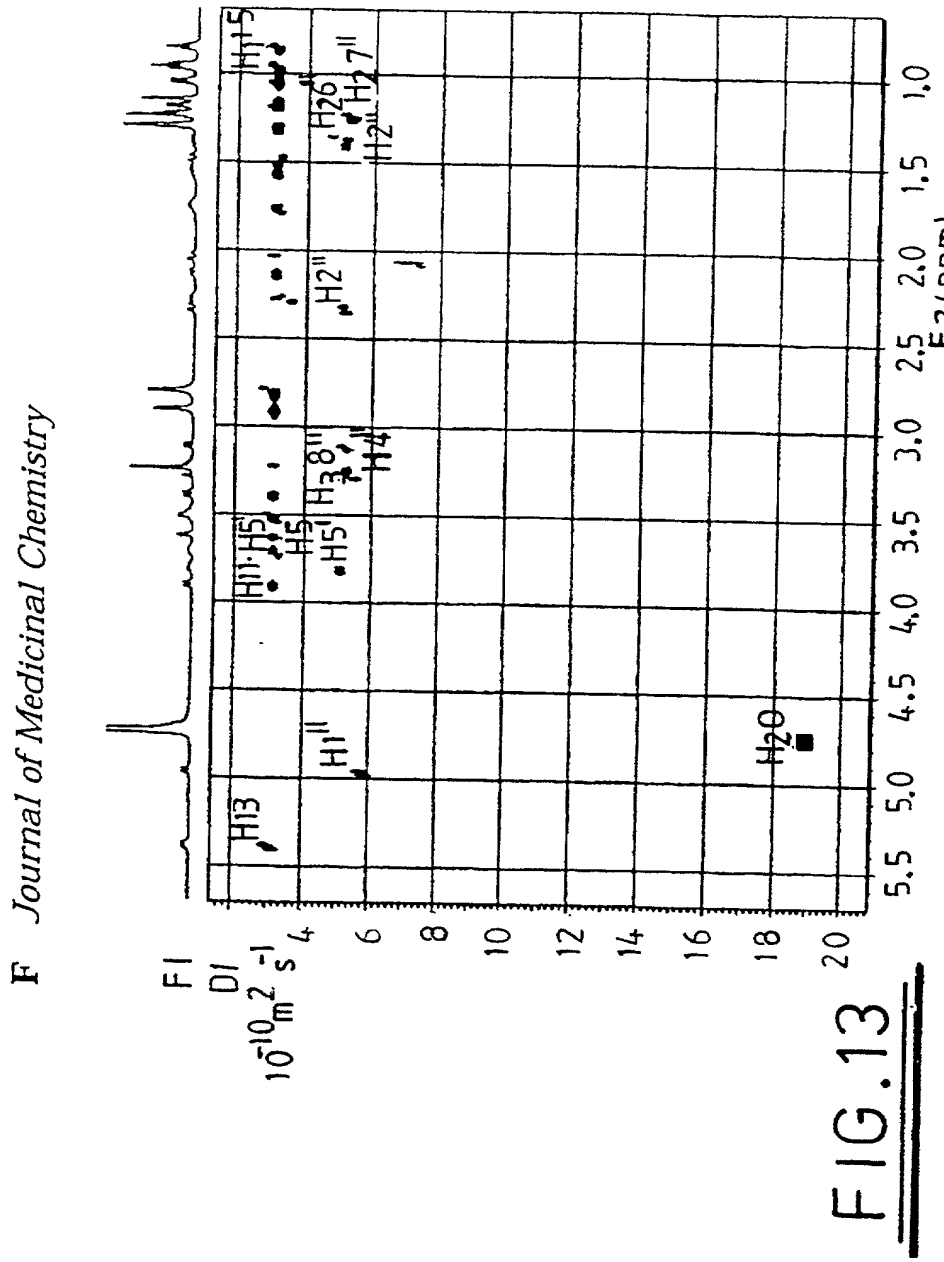


FIG.13

DOSY spectrum of the products of the degradation of erythromycin B in Britton–Robinson buffer at apparent pH 2.5 and 37 °C for 24 h.

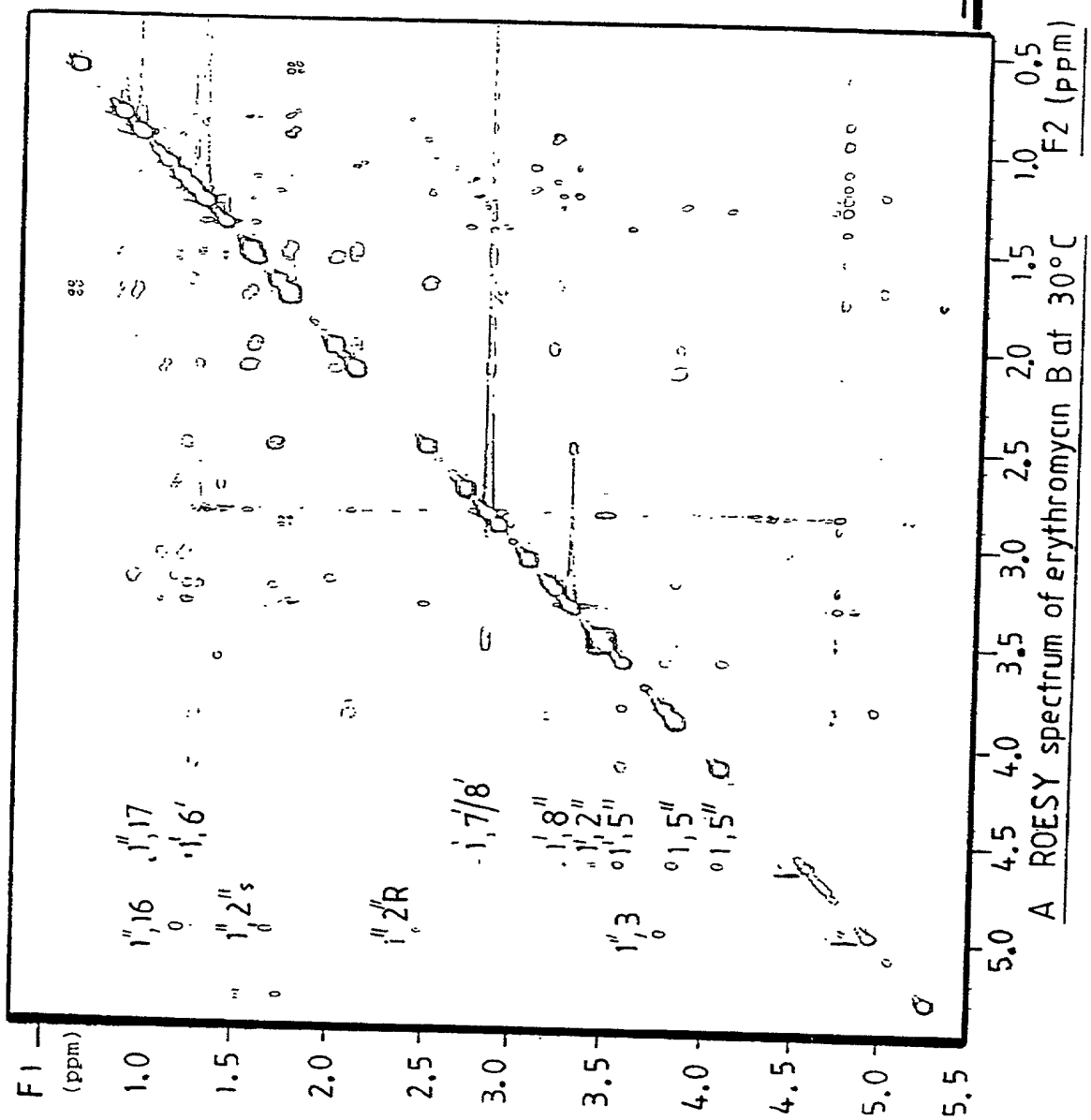

F1 (ppm)
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
1"16
1"17
1'6'
1"2"s
1"2R
1'7/8'
1"8"
1'2"
1'5"
1"3
1,5"
1,5"
1"
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
F2 (ppm)
A ROESY spectrum of erythromycin B at 30°C

FIG.14

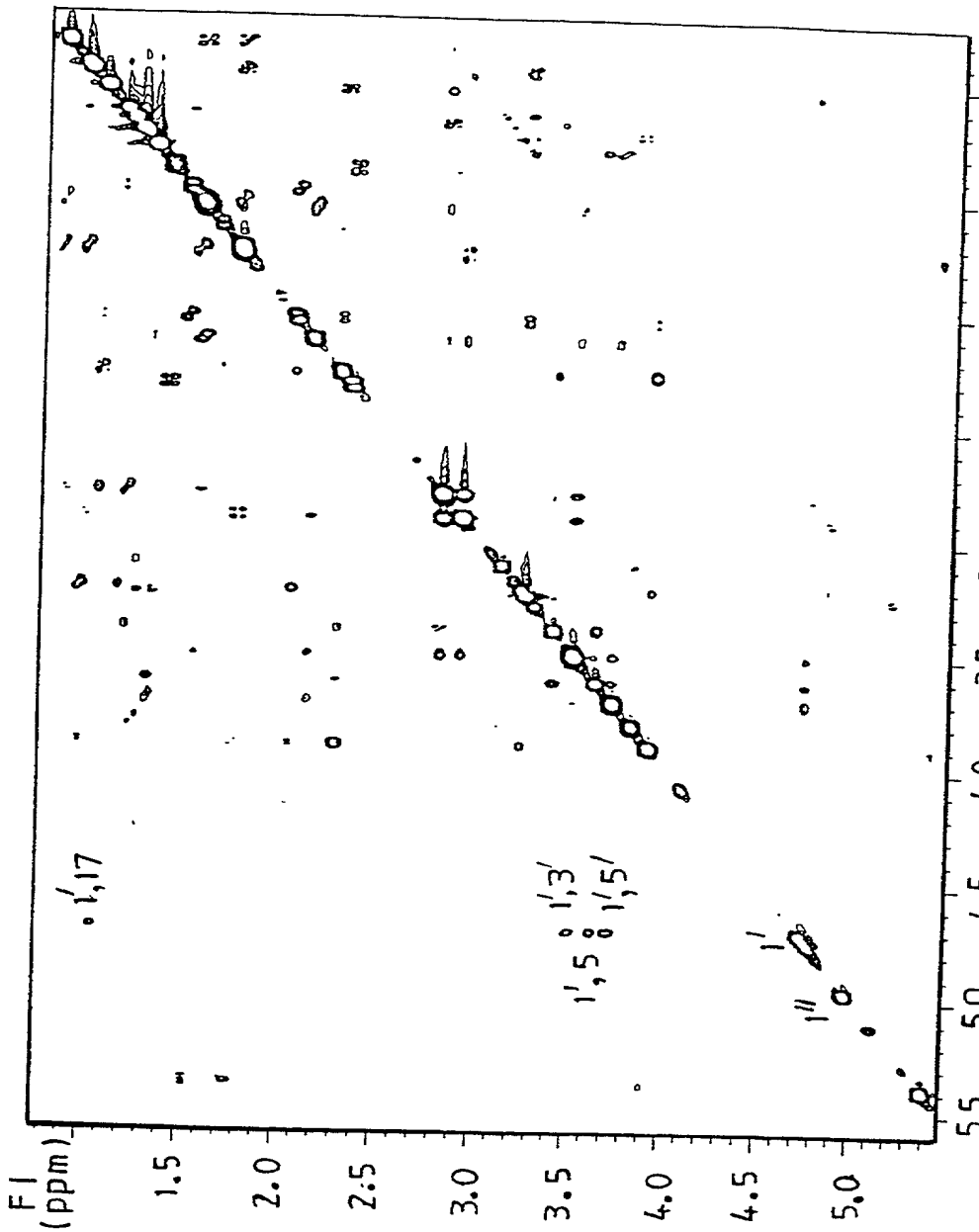


FIG.15

ROESY spectrum at 30°C from degradation mixtures of erythromycin B, containing 5-deB and cladinose

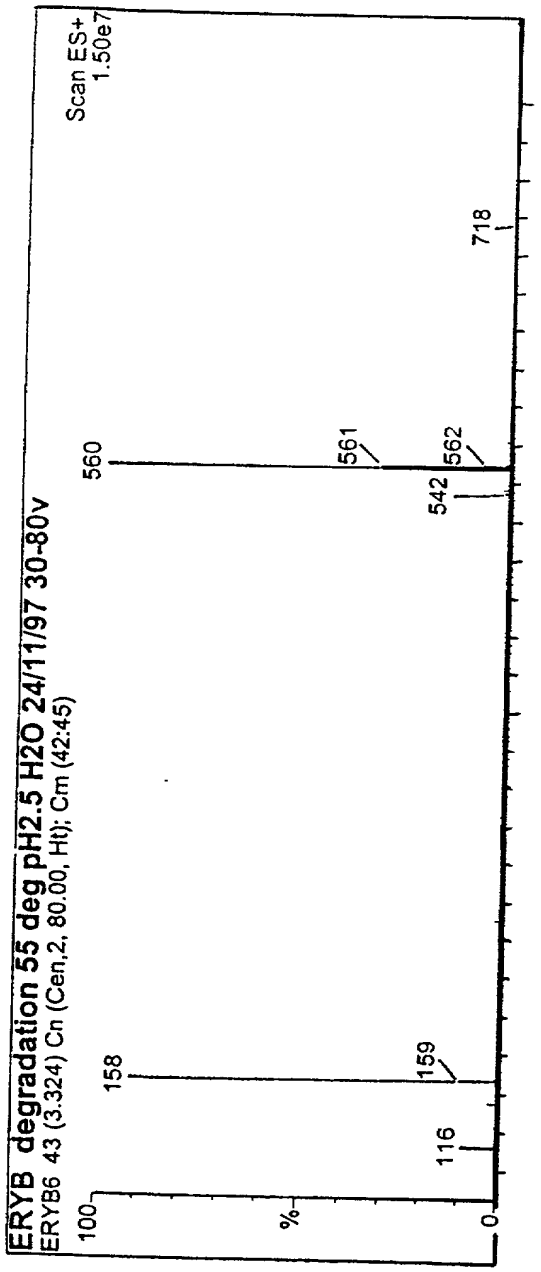
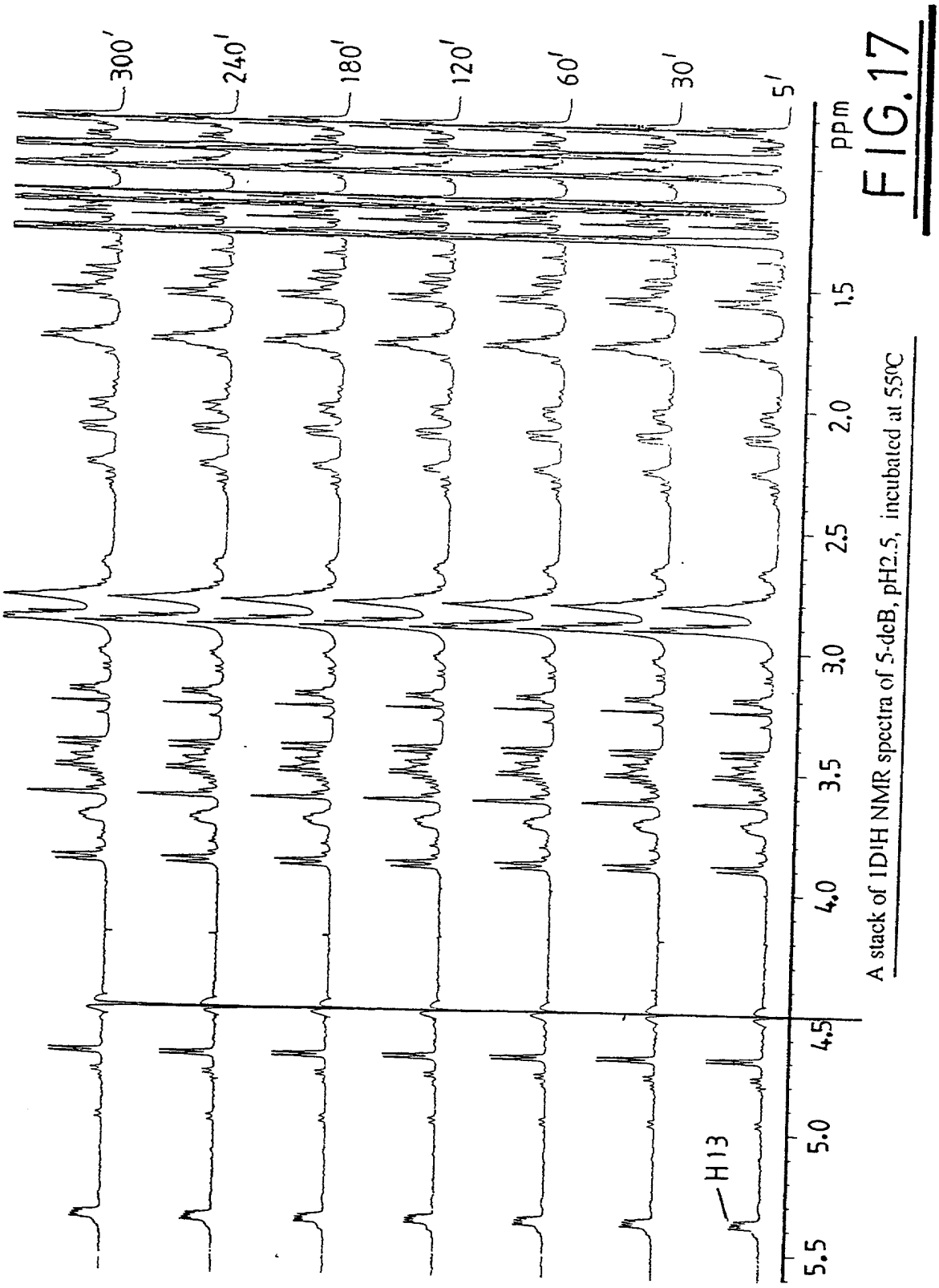


FIG. 16

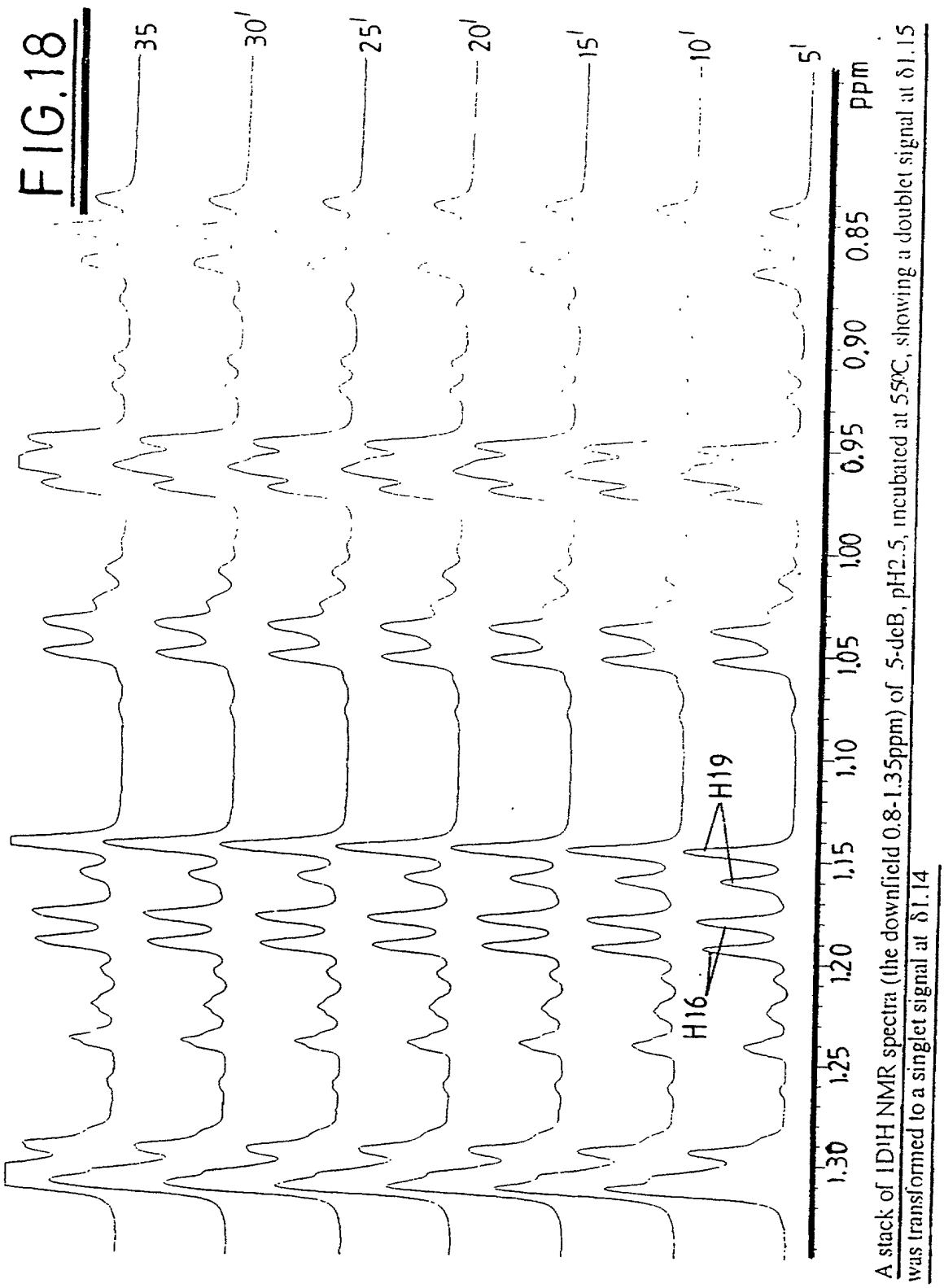
Electrospray-Mass spectrum (in positive mode) of erythromycin B (MW 717), 5-deB (MW 559) and cladinose (MW 157) in protiated buffer, pH 2.5. Please note that the actual mass of every compounds should be less 1 mass unit from the values shown in the spectrum.



A stack of 1D ^{1}H NMR spectra of 5-deB, pH2.5, incubated at 55°C

FIG.17

FIG.18



A stack of 1DIH NMR spectra (the downfield 0.8-1.35ppm) of 5-deB, pH2.5, incubated at 55°C, showing a doublet signal at δ1.15 was transformed to a singlet signal at δ1.14

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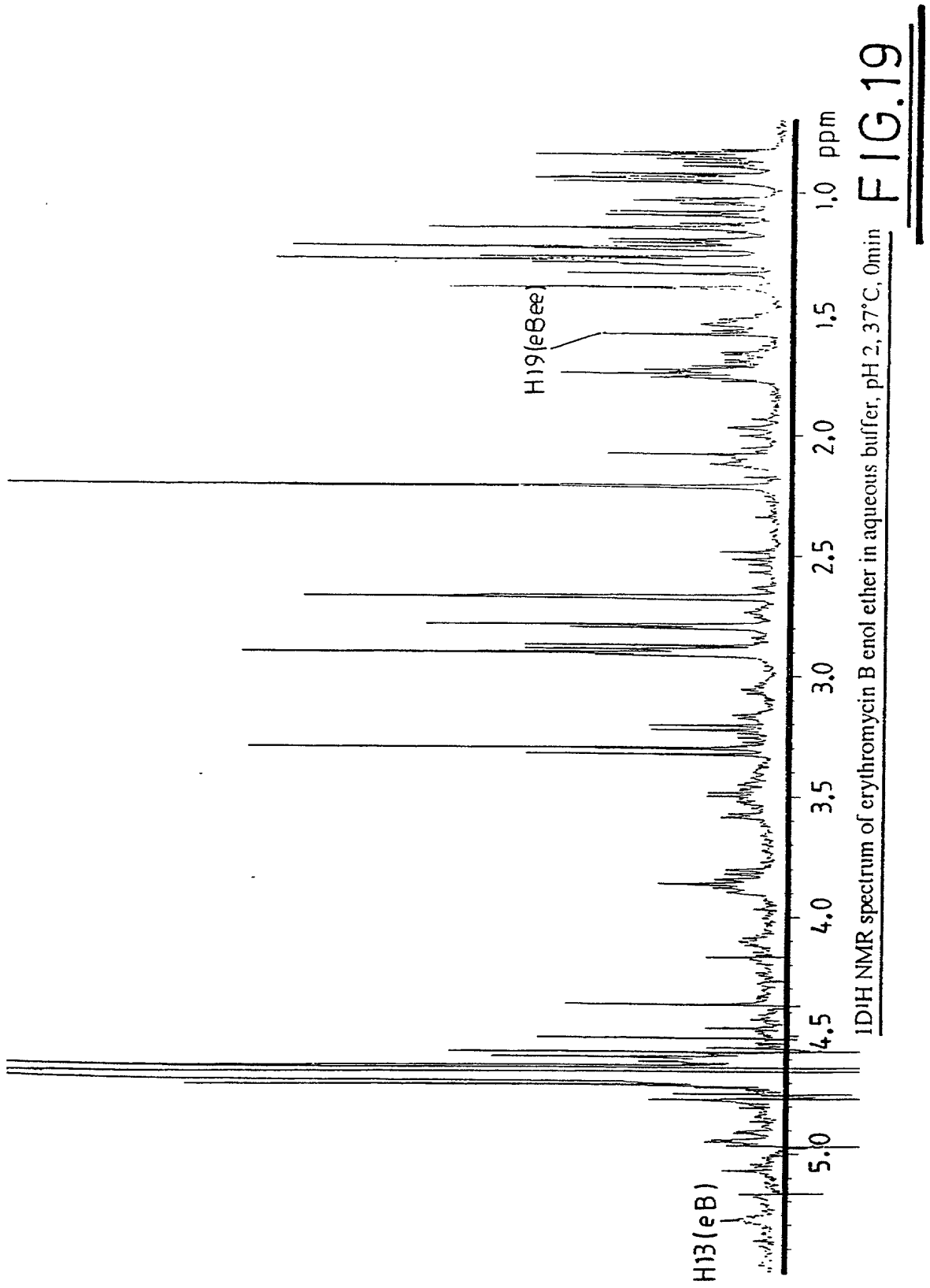
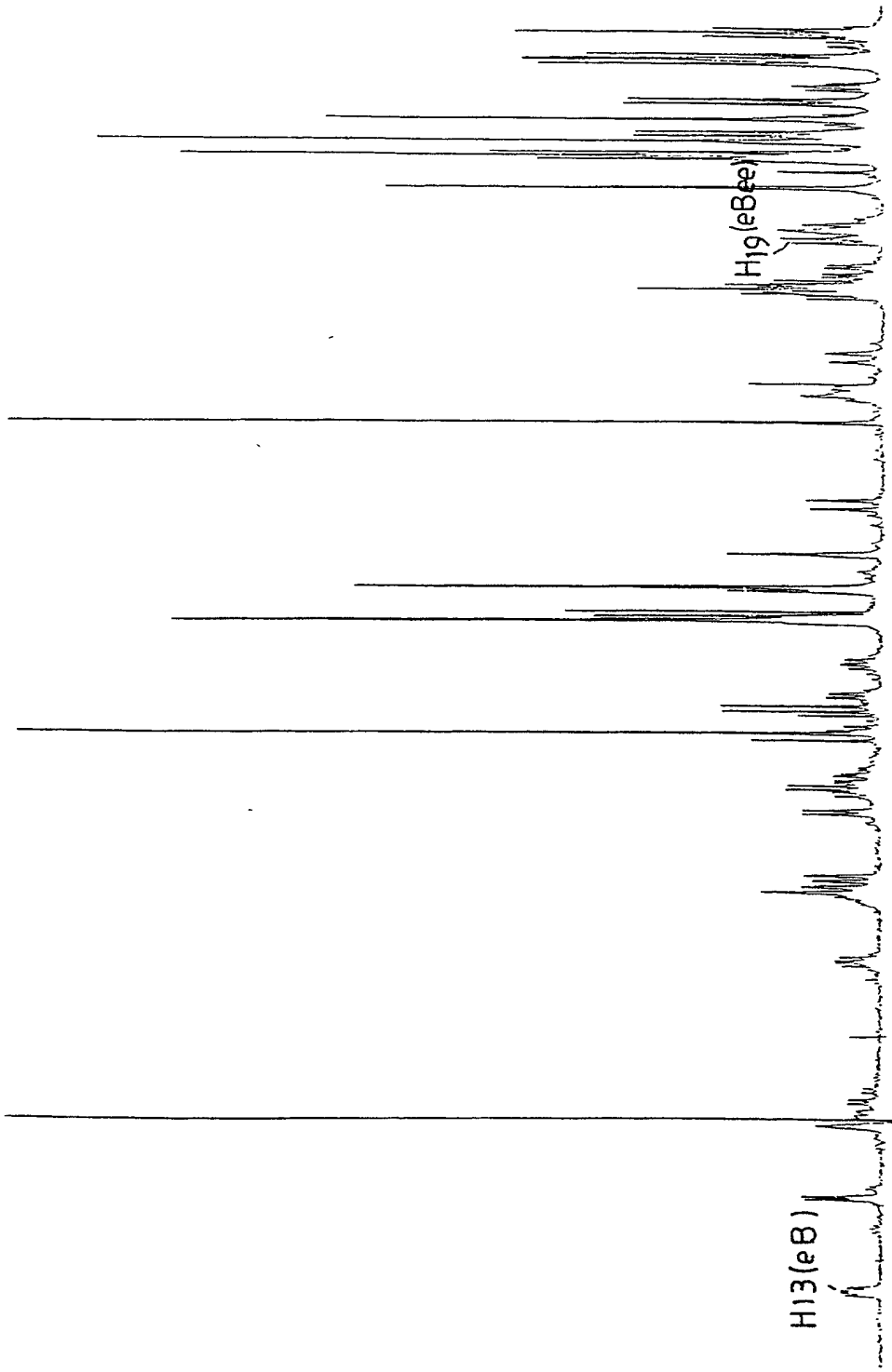


1D¹H NMR spectrum of erythromycin B enol ether in aqueous buffer, pH 2, 37°C, 0min FIG.19



1DIH NMR spectrum of erythromycin B enol ether in aqueous buffer, pH 2, degraded at 37°C for 10min

FIG. 20

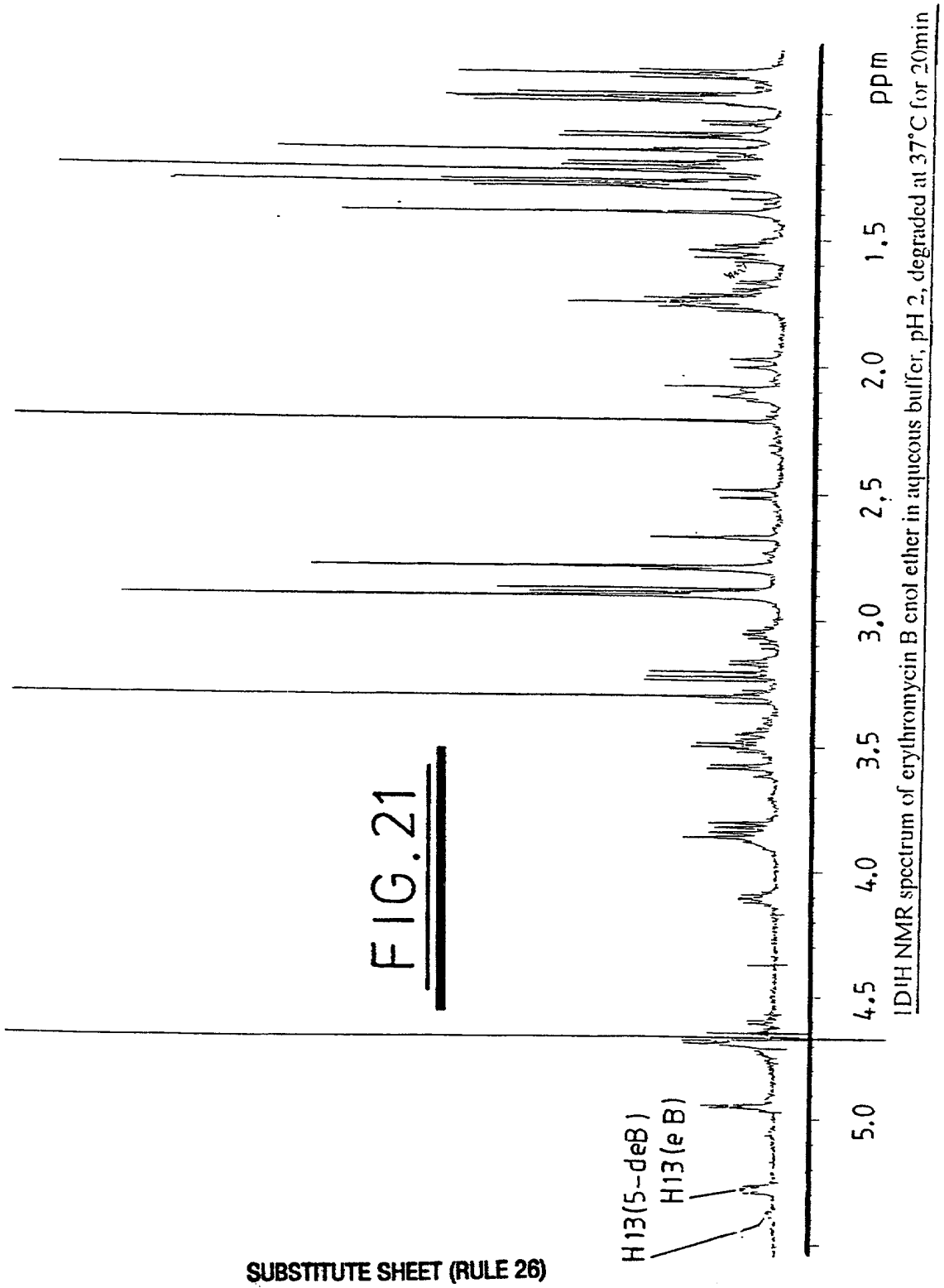


FIG. 21

1DIH NMR spectrum of erythromycin B enol ether in aqueous buffer, pH 2, degraded at 37°C for 20min

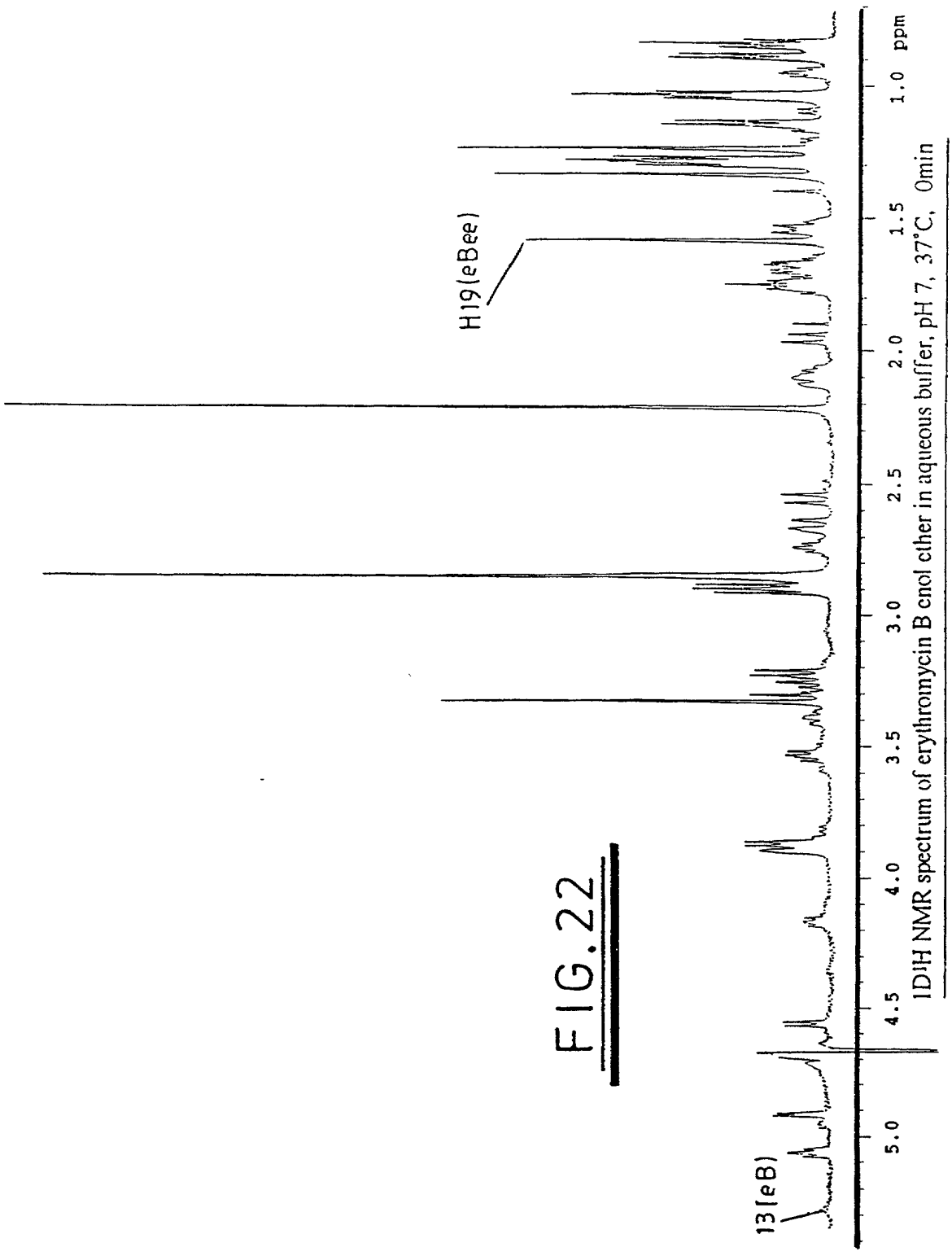


FIG.22

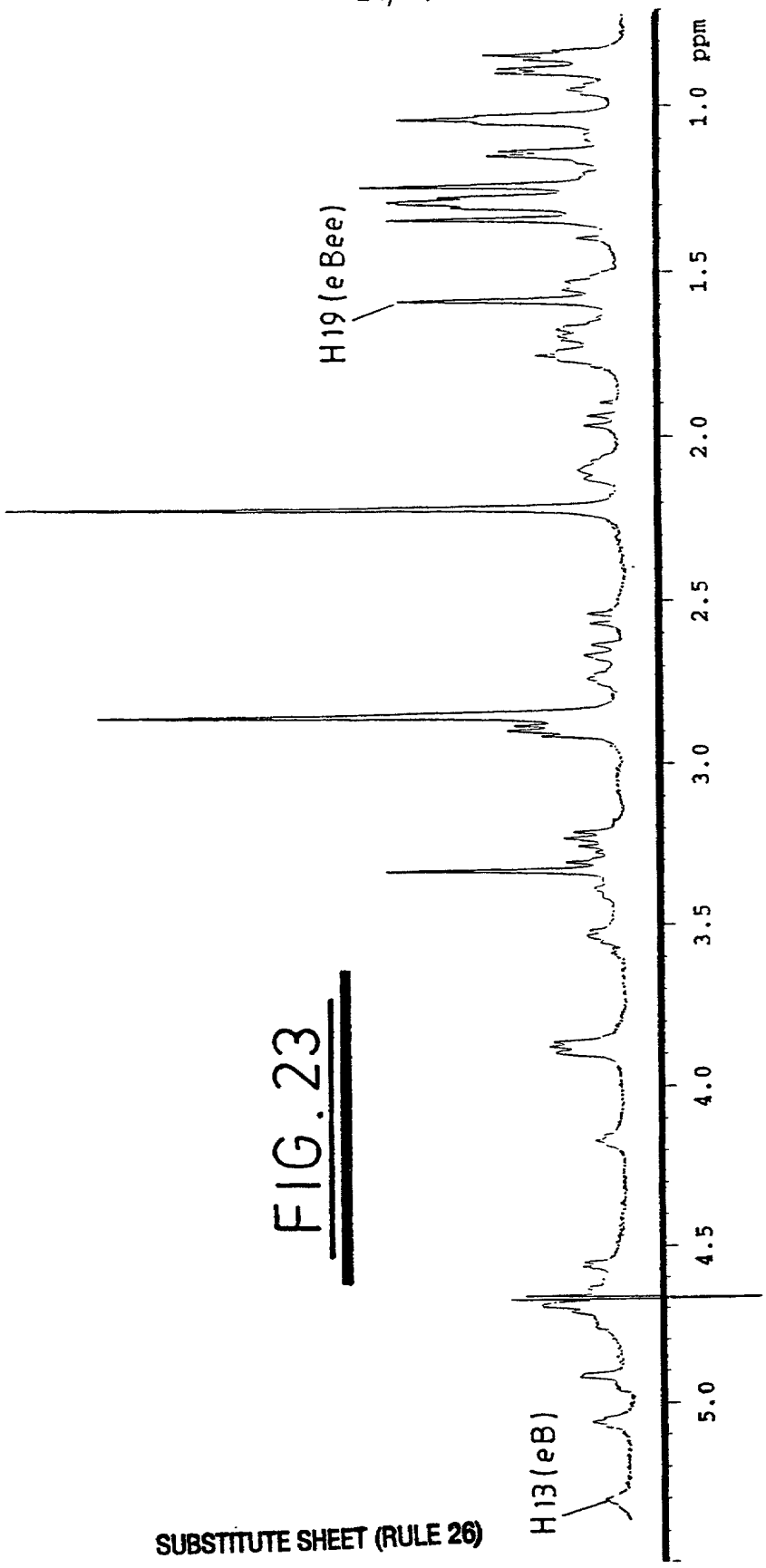


FIG. 23

1D1H NMR spectrum of erythromycin B enol ether in aqueous buffer, pH 7, degraded at 37°C for 80min

RULE 63 (37 C.F.R. 1.63)
INVENTORS DECLARATION FOR PATENT APPLICATION
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

As a below named inventor, I hereby declare that my residence, mailing address and citizenship are as stated below next to my name, and I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

ANTIBIOTIC AGENTS

the specification of which (check applicable box(s)):

- ☐ is attached hereto
- ☐ was filed on _____ as U.S. Application Serial No. _____ (Atty Dkt. No. 39-252)
- ☒ was filed as PCT international application No. PCT/GB00/02217 on 19 June 2000

and (if applicable to U.S. or PCT application) was amended on _____

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above. I acknowledge the duty to disclose to the Patent Office all information known to me to be material to patentability as defined in 37 C.F.R. 1.56. I hereby claim foreign priority benefits under 35 U.S.C. 119/365 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed or, if no priority is claimed, before the filing date of this application:

Priority Foreign Application(s):

Application Number	Country	Day/Month/Year Filed
9914346.3	Great Britain	19 June 1999

I hereby claim the benefit under 35 U.S.C. §119(e) of any United States provisional application(s) listed below.

Application Number	Date/Month/Year Filed

I hereby claim the benefit under 35 U.S.C. 120/365 of all prior United States and PCT international applications listed above or below:

Prior U.S./PCT Application(s): Application Serial No.	Day/Month/Year Filed	Status: patented pending, abandoned
PCT/GB00/02217	19 June 2000	

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon. And on behalf of the owner(s) hereof, I hereby appoint **NIXON & VANDERHYE P.C., 1100 North Glebe Rd., 8th Floor, Arlington, VA 22201-4714, telephone number (703) 816-4000 (to whom all communications are to be directed)**, and the following attorneys thereof (of the same address) individually and collectively owner's/owners' attorneys to prosecute this application and to transact all business in the Patent and Trademark Office connected therewith and with the resulting patent: Larry S. Nixon, 25640; Arthur R. Crawford, 25327; James T. Hosmer, 30184; Robert W. Faris, 31352; Richard G. Besha, 22770; Mark E. Nusbaum, 32348; Michael J. Keenan, 32106; Bryan H. Davidson, 30251; Stanley C. Spooner, 27393; Leonard C. Mitchard, 29009; Duane M. Byers, 33363; Jeffry H. Nelson, 30481; John R. Lastova, 33149; H. Warren Burnam, Jr., 29366; Mary J. Wilson, 32955; J. Scott Davidson, 33489; Alan M. Kagen, 36178; Robert A. Molan, 29834; B. J. Sadoff, 36663; James D. Berquist, 34776; Updeep S. Gill, 37334; Michael J. Shea, 34725; Donald L. Jackson, 41090; Michelle N. Lester, 32331; Frank P. Presta, 19828; Joseph S. Presta, 35329; Joseph A. Rhoa, 37515; Raymond Y. Mah, 41426; Chris Comuntzis, 31097; Gary T. Tanigawa, 43180. I also authorize Nixon & Vanderhye to delete any attorney names/numbers no longer with the firm and to act and rely solely on instructions directly communicated from the person, assignee, attorney, firm, or other organization sending instructions to Nixon & Vanderhye on behalf of the owner(s).

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☐ *See attached sheet(s) for additional inventor(s) information!!*